EXHIBIT B

Guidance for Industry

Sterile Drug Products
Produced by Aseptic Processing —
Current Good Manufacturing Practice

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Current Good Manufacturing Practice

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)
Office of Regulatory affairs (ORA)

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Aseptic Processing — Current Good Manufacturing Practice

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I.   INTRODUCTION

This guidance is intended to help manufacturers meet the requirements in the Agency's current good manufacturing practice (CGMP) regulations (21 CFR parts 210 and 211) when manufacturing sterile drug and biological products using aseptic processing. This guidance replaces the 1987 *Industry Guideline on Sterile Drug Products Produced by Aseptic Processing (Aseptic Processing Guideline)*. This revision updates and clarifies the 1987 guidance.

For sterile drug products subject to a new or abbreviated drug application (NDA or ANDA) or a biologic license application (BLA), this guidance document should be read in conjunction with the guidance on the content of sterile drug applications entitled *Guideline for the Submission of Documentation for Sterilization Process Validation in Applications for Human and Veterinary Drug Products* (Submission Guidance). The Submission Guidance describes the types of information and data that should be included in drug applications to demonstrate the efficacy of a manufacturer's sterilization process. This guidance compliments the Submission Guidance by describing procedures and practices that will help enable a sterile drug manufacturing facility to meet CGMP requirements relating, for example, to facility design, equipment suitability, process validation, and quality control.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

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[1] This guidance was developed by the Office of Compliance in the Center for Drug Evaluation and Research (CDER) in cooperation with the Center for Biologics Evaluation and Research (CBER) and the Office of Regulatory Affairs (ORA).

The text boxes included in this guidance include specific sections of parts 210 and 211 of the Code of Federal Regulations (CFR), which address current good manufacturing practice for drugs. The intent of including these quotes in the text boxes is to aid the reader by providing a portion of an applicable regulation being addressed in the guidance. The quotes included in the text boxes are not intended to be exhaustive. Readers of this document should reference the complete CFR to ensure that they have complied, in full, with all relevant sections of the regulations.

II.   BACKGROUND

This section describes briefly both the regulatory and technical reasons why the Agency is developing this guidance document.

A.   Regulatory Framework

This guidance pertains to current good manufacturing practice (CGMP) regulations (21 CFR parts 210 and 211) when manufacturing sterile drug and biological products using aseptic processing. Although the focus of this guidance is on CGMPs in 21 CFR 210 and 211, supplementary requirements for biological products are in 21 CFR 600-680. For biological products regulated under 21 CFR parts 600 through 680, §§ 210.2(a) and 211.1(b) provide that where it is impossible to comply with the applicable regulations in both parts 600 through 680 and parts 210 and 211, the regulation specifically applicable to the drug product in question shall supercede the more general regulations.

B.   Technical Framework

There are basic differences between the production of sterile drug products using aseptic processing and production using terminal sterilization.

Terminal sterilization usually involves filling and sealing product containers under high-quality environmental conditions. Products are filled and sealed in this type of environment to minimize the microbial and particulate content of the in-process product and to help ensure that the subsequent sterilization process is successful. In most cases, the product, container, and closure have low bioburden, but they are not sterile. The product in its final container is then subjected to a sterilization process such as heat or irradiation.

In an aseptic process, the drug product, container, and closure are first subjected to sterilization methods separately, as appropriate, and then brought together.[2] Because there is no process to sterilize the product in its final container, it is critical that containers be filled and sealed in an extremely high-quality environment. Aseptic processing involves more variables than terminal sterilization. Before aseptic assembly into a final product, the individual parts of the final product

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[2] Due to their nature, certain products are aseptically processed at an earlier stage in the process, or in their entirety. Cellular therapy products are an example. All components and excipients for these products are rendered sterile, and release of the final product is contingent on determination of sterility. See Appendix III.

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are generally subjected to various sterilization processes. For example, glass containers are subjected to dry heat; rubber closures are subjected to moist heat; and liquid dosage forms are subjected to filtration. Each of these manufacturing processes requires validation and control. Each process could introduce an error that ultimately could lead to the distribution of a contaminated product. Any manual or mechanical manipulation of the sterilized drug, components, containers, or closures prior to or during aseptic assembly poses the risk of contamination and thus necessitates careful control. A terminally sterilized drug product, on the other hand, undergoes final sterilization in a sealed container, thus limiting the possibility of error.[3]

Sterile drug manufacturers should have a keen awareness of the public health implications of distributing a nonsterile product. Poor CGMP conditions at a manufacturing facility can ultimately pose a life-threatening health risk to a patient.

III. SCOPE

This guidance document discusses selected issues and does not address all aspects of aseptic processing. For example, the guidance addresses primarily finished drug product CGMP issues while only limited information is provided regarding upstream bulk processing steps. This guidance updates the 1987 Aseptic Processing Guideline primarily with respect to personnel qualification, cleanroom design, process design, quality control, environmental monitoring, and review of production records. The use of isolators for aseptic processing is also discussed.

Although this guidance document discusses CGMP issues relating to the sterilization of components, containers, and closures, terminal sterilization of drug products is not addressed. It is a well-accepted principle that sterile drugs should be manufactured using aseptic processing only when terminal sterilization is not feasible. However, some final packaging may afford some unique and substantial advantage (e.g., some dual-chamber syringes) that would not be possible if terminal sterilization were employed. In such cases, a manufacturer can explore the option of adding adjunct processing steps to increase the level of sterility assurance.

A list of references that may be of value to the reader is included at the conclusion of this document.

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[3] Nearly all drugs recalled due to nonsterility or lack of sterility assurance in the period spanning 1980-2000 were produced via aseptic processing.

IV. BUILDINGS AND FACILITIES

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21 CFR 211.42(b) states, in part, that "The flow of components, drug product containers, closures, labeling, in-process materials, and drug products through the building or buildings shall be designed to prevent contamination."

21 CFR 211.42(c) states, in part, that "Operations shall be performed within specifically defined areas of adequate size. There shall be separate or defined areas or such other control systems for the firm's operations as are necessary to prevent contamination or mixups during the course of the following procedures: * * * (10) Aseptic processing, which includes as appropriate: (i) Floors, walls, and ceilings of smooth, hard surfaces that are easily cleanable; (ii) Temperature and humidity controls; (iii) An air supply filtered through high-efficiency particulate air filters under positive pressure, regardless of whether flow is laminar or nonlaminar; (iv) A system for monitoring environmental conditions; (v) A system for cleaning and disinfecting the room and equipment to produce aseptic conditions; (vi) A system for maintaining any equipment used to control the aseptic conditions."

21 CFR 211.46(b) states that "Equipment for adequate control over air pressure, micro-organisms, dust, humidity, and temperature shall be provided when appropriate for the manufacture, processing, packing, or holding of a drug product."

21 CFR 211.46(c) states, in part, that "Air filtration systems, including prefilters and particulate matter air filters, shall be used when appropriate on air supplies to production areas * * *."

21 CFR 211.63 states that "Equipment used in the manufacture, processing, packing, or holding of a drug product shall be of appropriate design, adequate size, and suitably located to facilitate operations for its intended use and for its cleaning and maintenance."

21 CFR 211.65(a) states that "Equipment shall be constructed so that surfaces that contact components, in-process materials, or drug products shall not be reactive, additive, or absorptive so as to alter the safety, identity, strength, quality, or purity of the drug product beyond the official or other established requirements."

21 CFR 211.67(a) states that "Equipment and utensils shall be cleaned, maintained, and sanitized at appropriate intervals to prevent malfunctions or contamination that would alter the safety, identity, strength, quality, or purity of the drug product beyond the official or other established requirements."

21 CFR 211.113(b) states that "Appropriate written procedures, designed to prevent microbiological contamination of drug products purporting to be sterile, shall be established and followed. Such procedures shall include validation of any sterilization process."

As provided for in the regulations, separate or defined areas of operation in an aseptic processing facility should be appropriately controlled to attain different degrees of air quality depending on the nature of the operation. Design of a given area involves satisfying microbiological and particle criteria as defined by the equipment, components, and products exposed, as well as the operational activities conducted in the area.

Clean area control parameters should be supported by microbiological and particle data obtained during qualification studies. Initial cleanroom qualification includes, in part, an assessment of air quality under as-built, static conditions. It is important for area qualification and classification to place most emphasis on data generated under dynamic conditions (i.e., with personnel present, equipment in place, and operations ongoing). An adequate aseptic processing facility monitoring program also will assess conformance with specified clean area classifications under dynamic conditions on a routine basis.

The following table summarizes clean area air classifications and recommended action levels of microbiological quality (Ref. 1).

TABLE 1- Air Classifications[a]

Clean Area Classification (0.5 um particles/ft³)	ISO Designation[b]	≥ 0.5 μm particles/m³	Microbiological Active Air Action Levels[c] (cfu/m³)	Microbiological Settling Plates Action Levels[c,d] (diam. 90mm; cfu/4 hours)
100	5	3,520	1[e]	1[e]
1000	6	35,200	7	3
10,000	7	352,000	10	5
100,000	8	3,520,000	100	50

a- All classifications based on data measured in the vicinity of exposed materials/articles during periods of activity.

b- ISO 14644-1 designations provide uniform particle concentration values for cleanrooms in multiple industries. An ISO 5 particle concentration is equal to Class 100 and approximately equals EU Grade A.

c- Values represent recommended levels of environmental quality. You may find it appropriate to establish alternate microbiological action levels due to the nature of the operation or method of analysis.

d- The additional use of settling plates is optional.

e- Samples from Class 100 (ISO 5) environments should normally yield no microbiological contaminants.

Two clean areas are of particular importance to sterile drug product quality: the critical area and the supporting clean areas associated with it.

A.   Critical Area – Class 100 (ISO 5)

A critical area is one in which the sterilized drug product, containers, and closures are exposed to environmental conditions that must be designed to maintain product sterility (§ 211.42(c)(10)). Activities conducted in such areas include manipulations (e.g., aseptic connections, sterile ingredient additions) of sterile materials prior to and during filling and closing operations.

This area is critical because an exposed product is vulnerable to contamination and will not be subsequently sterilized in its immediate container. To maintain product sterility, it is essential that the environment in which aseptic operations (e.g., equipment setup, filling) are conducted be controlled and maintained at an appropriate quality. One aspect of environmental quality is the particle content of the air. Particles are significant because they can enter a product as an extraneous contaminant, and can also contaminate it biologically by acting as a vehicle for microorganisms (Ref. 2). Appropriately designed air handling systems minimize particle content of a critical area.

Air in the immediate proximity of exposed sterilized containers/closures and filling/closing operations would be of appropriate particle quality when it has a per-cubic-meter particle count

of no more than 3520 in a size range of 0.5 μm and larger when counted at representative locations normally not more than 1 foot away from the work site, within the airflow, and during filling/closing operations. This level of air cleanliness is also known as Class 100 (ISO 5).

We recommend that measurements to confirm air cleanliness in critical areas be taken at sites where there is most potential risk to the exposed sterilized product, containers, and closures. The particle counting probe should be placed in an orientation demonstrated to obtain a meaningful sample. Regular monitoring should be performed during each production shift. We recommend conducting nonviable particle monitoring with a remote counting system. These systems are capable of collecting more comprehensive data and are generally less invasive than portable particle counters. See Section X.E. for additional guidance on particle monitoring.

Some operations can generate high levels of product (e.g., powder) particles that, by their nature, do not pose a risk of product contamination. It may not, in these cases, be feasible to measure air quality within the one-foot distance and still differentiate background levels of particles from air contaminants. In these instances, air can be sampled in a manner that, to the extent possible, characterizes the true level of extrinsic particle contamination to which the product is exposed. Initial qualification of the area under dynamic conditions without the actual filling function provides some baseline information on the non-product particle generation of the operation.

HEPA-filtered[4] air should be supplied in critical areas at a velocity sufficient to sweep particles away from the filling/closing area and maintain unidirectional airflow during operations. The velocity parameters established for each processing line should be justified and appropriate to maintain unidirectional airflow and air quality under dynamic conditions within the critical area (Ref. 3).[5]

Proper design and control prevents turbulence and stagnant air in the critical area. Once relevant parameters are established, it is crucial that airflow patterns be evaluated for turbulence or eddy currents that can act as a channel or reservoir for air contaminants (e.g., from an adjoining lower classified area). In situ air pattern analysis should be conducted at the critical area to demonstrate unidirectional airflow and sweeping action over and away from the product under dynamic conditions. The studies should be well documented with written conclusions, and include evaluation of the impact of aseptic manipulations (e.g., interventions) and equipment design. Videotape or other recording mechanisms have been found to be useful aides in assessing airflow initially as well as facilitating evaluation of subsequent equipment configuration changes. It is important to note that even successfully qualified systems can be compromised by poor operational, maintenance, or personnel practices.

Air monitoring samples of critical areas should normally yield no microbiological contaminants. We recommend affording appropriate investigative attention to contamination occurrences in this environment.

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[4]High Efficiency Particulate Air filter

[5] A velocity of 0.45 meters/second (90 feet per minute) has generally been established, with a range of plus or minus 20 percent around the setpoint. Higher velocities may be appropriate in operations generating high levels of particulates.

B. Supporting Clean Areas

Supporting clean areas can have various classifications and functions. Many support areas function as zones in which nonsterile components, formulated products, in-process materials, equipment, and container/closures are prepared, held, or transferred. These environments are soundly designed when they minimize the level of particle contaminants in the final product and control the microbiological content (bioburden) of articles and components that are subsequently sterilized.

The nature of the activities conducted in a supporting clean area determines its classification. FDA recommends that the area immediately adjacent to the aseptic processing line meet, at a minimum, Class 10,000 (ISO 7) standards (see Table 1) under dynamic conditions. Manufacturers can also classify this area as Class 1,000 (ISO 6) or maintain the entire aseptic filling room at Class 100 (ISO 5). An area classified at a Class 100,000 (ISO 8) air cleanliness level is appropriate for less critical activities (e.g., equipment cleaning).

C. Clean Area Separation

An essential part of contamination prevention is the adequate separation of areas of operation. To maintain air quality, it is important to achieve a proper airflow from areas of higher cleanliness to adjacent less clean areas. It is vital for rooms of higher air cleanliness to have a substantial positive pressure differential relative to adjacent rooms of lower air cleanliness. For example, a positive pressure differential of at least 10-15 Pascals (Pa)[6] should be maintained between adjacent rooms of differing classification (with doors closed). When doors are open, outward airflow should be sufficient to minimize ingress of contamination, and it is critical that the time a door can remain ajar be strictly controlled (Ref. 4).

In some cases, the aseptic processing room and adjacent cleanrooms have the same classification. Maintaining a pressure differential (with doors closed) between the aseptic processing room and these adjacent rooms can provide beneficial separation. In any facility designed with an unclassified room adjacent to the aseptic processing room, a substantial overpressure (e.g., at least 12.5 Pa) from the aseptic processing room should be maintained at all times to prevent contamination. If this pressure differential drops below the minimum limit, it is important that the environmental quality of the aseptic processing room be restored and confirmed.

The Agency recommends that pressure differentials between cleanrooms be monitored continuously throughout each shift and frequently recorded. All alarms should be documented and deviations from established limits should be investigated.

Air change rate is another important cleanroom design parameter. For Class 100,000 (ISO 8) supporting rooms, airflow sufficient to achieve at least 20 air changes per hour is typically acceptable. Significantly higher air change rates are normally needed for Class 10,000 and Class 100 areas.

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[6] Equal to 0.04-0.06 inches of water gauge.

A suitable facility monitoring system will rapidly detect atypical changes that can compromise the facility's environment. An effective system facilitates restoration of operating conditions to established, qualified levels before reaching action levels. For example, pressure differential specifications should enable prompt detection (i.e., alarms) of an emerging low pressure problem to preclude ingress of unclassified air into a classified room.

D.   Air Filtration

1.   Membrane

A compressed gas should be of appropriate purity (e.g., free from oil) and its microbiological and particle quality after filtration should be equal to or better than that of the air in the environment into which the gas is introduced. Compressed gases such as air, nitrogen, and carbon dioxide are often used in cleanrooms and are frequently employed in purging or overlaying.

Membrane filters can be used to filter a compressed gas to meet an appropriate high-quality standard. These filters are often used to produce a sterile compressed gas to conduct operations involving sterile materials, such as components and equipment. For example, we recommend that sterile membrane filters be used for autoclave air lines, lyophilizer vacuum breaks, and tanks containing sterilized materials. Sterilized holding tanks and any contained liquids should be held under positive pressure or appropriately sealed to prevent microbial contamination. Safeguards should be in place to prevent a pressure change that can result in contamination due to back flow of nonsterile air or liquid.

Gas filters (including vent filters) should be dry. Condensate on a gas filter can cause blockage during use or allow for the growth of microorganisms. Use of hydrophobic filters, as well as application of heat to these filters where appropriate, prevents problematic moisture residues. We recommend that filters that serve as sterile boundaries or supply sterile gases that can affect product be integrity tested upon installation and periodically thereafter (e.g., end of use). Integrity tests are also recommended after activities that may damage the filter. Integrity test failures should be investigated, and filters should be replaced at appropriate, defined intervals.

2.   High-Efficiency Particulate Air (HEPA)[7]

HEPA filter integrity should be maintained to ensure aseptic conditions. Leak testing should be performed at installation to detect integrity breaches around the sealing gaskets, through the frames, or through various points on the filter media. Thereafter, leak tests should be performed at suitable time intervals for HEPA filters in the aseptic processing facility. For example, such testing should be performed twice a year for the aseptic processing room. Additional testing may be appropriate when air quality is found to be unacceptable, facility renovations might be the cause of disturbances to ceiling or wall structures, or as part of an investigation into a media fill or drug product sterility failure. Among the filters that should be leak tested are those installed in dry heat depyrogenation tunnels and ovens commonly used to depyrogenate glass vials. Where justified, alternate methods can be used to test HEPA filters in the hot zones of these tunnels and ovens.

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[7] The same broad principles can be applied to ULPA filters.

Any aerosol used for challenging a HEPA filter should meet specifications for critical physicochemical attributes such as viscosity. Dioctylphthalate (DOP) and poly-alpha-olefin (PAO) are examples of appropriate leak testing aerosols. Some aerosols are problematic because they pose the risk of microbial contamination of the environment being tested. Accordingly, the evaluation of any alternative aerosol involves ensuring it does not promote microbial growth.

There is a major difference between *filter leak testing* and *efficiency testing*. An efficiency test is a general test used to determine the rating of the filter.[8] An intact HEPA filter should be capable of retaining at least 99.97 percent of particulates greater than 0.3 μm in diameter.

The purpose of performing regularly scheduled leak tests, on the other hand, is to detect leaks from the filter media, filter frame, or seal. The challenge involves use of a polydispersed aerosol usually composed of particles with a light-scattering mean droplet diameter in the submicron size range,[9] including a sufficient number of particles at approximately 0.3 μm. Performing a leak test without introducing a sufficient upstream challenge of particles of known size upstream of the filter is ineffective for detecting leaks. It is important to introduce an aerosol upstream of the filter in a concentration that is appropriate for the accuracy of the aerosol photometer. The leak test should be done in place, and the filter face scanned on the downstream side with an appropriate photometer probe, at a sampling rate of at least one cubic foot per minute. The downstream leakage measured by the probe should then be calculated as a percent of the upstream challenge. An appropriate scan should be conducted on the entire filter face and frame, at a position about one to two inches from the face of the filter. This comprehensive scanning of HEPA filters should be fully documented.

A single probe reading equivalent to 0.01 percent of the upstream challenge would be considered as indicative of a significant leak and calls for replacement of the HEPA filter or, when appropriate, repair in a limited area. A subsequent confirmatory retest should be performed in the area of any repair.

HEPA filter leak testing alone is insufficient to monitor filter performance. It is important to conduct periodic monitoring of filter attributes such as uniformity of velocity across the filter (and relative to adjacent filters). Variations in velocity can cause turbulence that increases the possibility of contamination. Velocities of unidirectional air should be measured 6 inches from the filter face and at a defined distance proximal to the work surface for HEPA filters in the critical area. Velocity monitoring at suitable intervals can provide useful data on the critical area in which aseptic processing is performed. The measurements should correlate to the velocity range established at the time of in situ air pattern analysis studies. HEPA filters should be replaced when nonuniformity of air velocity across an area of the filter is detected or airflow patterns may be adversely affected.

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[8] The efficiency test uses a monodispersed aerosol of 0.3 micron sized particles and assesses filter media. Downstream readings represent an average over the entire filter surface. Efficiency tests are not intended to test for filter leaks.

[9] Although the mean is normally less than one micron, it is greater than 0.3μm.

Although contractors often provide these services, drug manufacturers are responsible for ensuring that equipment specifications, test methods, and acceptance criteria are defined, and that these essential certification activities are conducted satisfactorily.

E.   Design

Note: The design concepts discussed within this section are not intended to be exhaustive. Other appropriate technologies that achieve increased sterility assurance are also encouraged.

Aseptic processes are designed to minimize exposure of sterile articles to the potential contamination hazards of the manufacturing operation. Limiting the duration of exposure of sterile product elements, providing the highest possible environmental control, optimizing process flow, and designing equipment to prevent entrainment of lower quality air into the Class 100 (ISO 5) clean area are essential to achieving high assurance of sterility (Ref. 4).

Both personnel and material flow should be optimized to prevent unnecessary activities that could increase the potential for introducing contaminants to exposed product, container-closures, or the surrounding environment. The layout of equipment should provide for ergonomics that optimize comfort and movement of operators. The number of personnel in an aseptic processing room should be minimized. The flow of personnel should be designed to limit the frequency with which entries and exits are made to and from an aseptic processing room and, most significant, its critical area. Regarding the latter, the number of transfers into the critical area of a traditional cleanroom, or an isolator, should be minimized. To prevent changes in air currents that introduce lower quality air, movement adjacent to the critical area should be appropriately restricted.

Any intervention or stoppage during an aseptic process can increase the risk of contamination. The design of equipment used in aseptic processing should limit the number and complexity of aseptic interventions by personnel. For example, personnel intervention can be reduced by integrating an on-line weight check device, thus eliminating a repeated manual activity within the critical area. Rather than performing an aseptic connection, sterilizing the preassembled connection using sterilize-in-place (SIP) technology also can eliminate a significant aseptic manipulation. Automation of other process steps, including the use of technologies such as robotics, can further reduce risk to the product.

Products should be transferred under appropriate cleanroom conditions. For example, lyophilization processes include transfer of aseptically filled product in partially sealed containers. To prevent contamination, a partially closed sterile product should be transferred only in critical areas.[10] Facility design should ensure that the area between a filling line and the lyophilizer provide for Class 100 (ISO 5) protection. Transport and loading procedures should afford the same protection.

The sterile drug product and its container-closures should be protected by equipment of suitable design. Carefully designed curtains and rigid plastic shields are among the barriers that can be

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[10] Appropriately designed transfer equipment provides these conditions and can be qualified for this purpose.

used in appropriate locations to achieve segregation of the aseptic processing line. Use of an isolator system further enhances product protection (see Appendix 1).

Due to the interdependence of the various rooms that make up an aseptic processing facility, it is essential to carefully define and control the dynamic interactions permitted between cleanrooms. Use of a double-door or integrated sterilizer helps ensure direct product flow, often from a lower to a higher classified area. Airlocks and interlocking doors will facilitate better control of air balance throughout the aseptic processing facility. Airlocks should be installed between the aseptic manufacturing area entrance and the adjoining unclassified area. Other interfaces such as personnel transitions or material staging areas are appropriate locations for air locks. It is critical to adequately control material (e.g., in-process supplies, equipment, utensils) as it transfers from lesser to higher classified clean areas to prevent the influx of contaminants. For example, written procedures should address how materials are to be introduced into the aseptic processing room to ensure that room conditions remain uncompromised. In this regard, materials should be disinfected according to appropriate procedures or, when used in critical areas, rendered sterile by a suitable method.

If stoppered vials exit an aseptic processing zone or room prior to capping, appropriate assurances should be in place to safeguard the product, such as local protection until completion of the crimping step. Use of devices for on-line detection of improperly seated stoppers can provide additional assurance.

Cleanrooms are normally designed as functional units with specific purposes. The materials of construction of cleanrooms ensure ease of cleaning and sanitizing. Examples of adequate design features include seamless and rounded floor to wall junctions as well as readily accessible corners. Floors, walls, and ceilings should be constructed of smooth, hard surfaces that can be easily cleaned. Ceilings and associated HEPA filter banks should be designed to protect sterile materials from contamination. Cleanrooms also should not contain unnecessary equipment, fixtures, or materials.

Processing equipment and systems should be equipped with sanitary fittings and valves. With rare exceptions, drains are considered inappropriate for classified areas of the aseptic processing facility other than Class 100,000 (ISO 8) areas. It is essential that any drain installed in an aseptic processing facility be of suitable design.

Equipment should be appropriately designed (§ 211.63) to facilitate ease of sterilization. It is also important to ensure ease of installation to facilitate aseptic setup. The effect of equipment design on the cleanroom environment should be addressed. Horizontal surfaces or ledges that accumulate particles should be avoided. Equipment should not obstruct airflow and, in critical areas, its design should not disturb unidirectional airflow.

Deviation or change control systems should address atypical conditions posed by shutdown of air handling systems or other utilities, and the impact of construction activities on facility control. Written procedures should address returning a facility to operating conditions following a shutdown.

V. PERSONNEL TRAINING, QUALIFICATION, & MONITORING

21 CFR 211.22(a) states that "There shall be a quality control unit that shall have the responsibility and authority to approve or reject all components, drug product containers, closures, in-process materials, packaging material, labeling, and drug products, and the authority to review production records to assure that no errors have occurred or, if errors have occurred, that they have been fully investigated. The quality control unit shall be responsible for approving or rejecting drug products manufactured, processed, packed, or held under contract by another company."

21 CFR 211.22(c) states that "The quality control unit shall have the responsibility for approving or rejecting all procedures or specifications impacting on the identity, strength, quality, and purity of the drug product."

21 CFR 211.25(a) states that "Each person engaged in the manufacture, processing, packing, or holding of a drug product shall have education, training, and experience, or any combination thereof, to enable that person to perform the assigned functions. Training shall be in the particular operations that the employee performs and in current good manufacturing practice (including the current good manufacturing practice regulations in this chapter and written procedures required by these regulations) as they relate to the employee's functions. Training in current good manufacturing practice shall be conducted by qualified individuals on a continuing basis and with sufficient frequency to assure that employees remain familiar with CGMP requirements applicable to them."

21 CFR 211.25(b) states that "Each person responsible for supervising the manufacture, processing, packing, or holding of a drug product shall have the education, training, and experience, or any combination thereof, to perform assigned functions in such a manner as to provide assurance that the drug product has the safety, identity, strength, quality, and purity that it purports or is represented to possess."

21 CFR 211.25(c) states that "There shall be an adequate number of qualified personnel to perform and supervise the manufacture, processing, packing, or holding of each drug product."

21 CFR 211.28(a) states that "Personnel engaged in the manufacture, processing, packing, or holding of a drug product shall wear clean clothing appropriate for the duties they perform. Protective apparel, such as head, face, hand, and arm coverings, shall be worn as necessary to protect drug products from contamination."

21 CFR 211.28(b) states that "Personnel shall practice good sanitation and health habits."

21 CFR 211.28(c) states that "Only personnel authorized by supervisory personnel shall enter those areas of the buildings and facilities designated as limited-access areas."

21 CFR 211.28(d) states that "Any person shown at any time (either by medical examination or supervisory observation) to have an apparent illness or open lesions that may adversely affect the safety or quality of drug products shall be excluded from direct contact with components, drug product containers, closures, in-process materials, and drug products until the condition is corrected or determined by competent medical personnel not to jeopardize the safety or quality of drug products. All personnel shall be instructed to report to supervisory personnel any health conditions that may have an adverse effect on drug products."

21 CFR 211.42(c) states, in part, that "Operations shall be performed within specifically defined areas of adequate size. There shall be separate or defined areas or such other control systems for the firm's operations as are necessary to prevent contamination or mixups during the course of the following procedures: * * * (10) Aseptic processing, which includes as appropriate: * * * (iv) A system for monitoring environmental conditions * * *."

21 CFR 211.113(b) states that "Appropriate written procedures, designed to prevent microbiological contamination of drug products purporting to be sterile, shall be established and followed. Such procedures shall include validation of any sterilization process."

A.   Personnel

A well-designed, maintained, and operated aseptic process minimizes personnel intervention. As operator activities increase in an aseptic processing operation, the risk to finished product sterility also increases. To ensure maintenance of product sterility, it is critical for operators involved in aseptic activities to use aseptic technique at all times.

Appropriate training should be conducted before an individual is permitted to enter the aseptic manufacturing area. Fundamental training topics should include aseptic technique, cleanroom behavior, microbiology, hygiene, gowning, patient safety hazards posed by a nonsterile drug product, and the specific written procedures covering aseptic manufacturing area operations. After initial training, personnel should participate regularly in an ongoing training program. Supervisory personnel should routinely evaluate each operator's conformance to written procedures during actual operations. Similarly, the quality control unit should provide regular oversight of adherence to established, written procedures and aseptic technique during manufacturing operations.

Some of the techniques aimed at maintaining sterility of sterile items and surfaces include:

- Contact sterile materials only with sterile instruments

Sterile instruments should always be used in the handling of sterilized materials. Between uses, sterile instruments should be held under Class 100 (ISO 5) conditions and maintained in a manner that prevents contamination (e.g., placed in sterilized containers). Instruments should be replaced as necessary throughout an operation.

After initial gowning, sterile gloves should be regularly sanitized or changed, as appropriate, to minimize the risk of contamination. Personnel should not directly contact sterile products, containers, closures, or critical surfaces with any part of their gown or gloves.

- Move slowly and deliberately

Rapid movements can create unacceptable turbulence in a critical area. Such movements disrupt the unidirectional airflow, presenting a challenge beyond intended cleanroom design and control parameters. The principle of slow, careful movement should be followed throughout the cleanroom.

- Keep the entire body out of the path of unidirectional airflow

Unidirectional airflow design is used to protect sterile equipment surfaces, container-closures, and product. Disruption of the path of unidirectional flow air in the critical area can pose a risk to product sterility.

- Approach a necessary manipulation in a manner that does not compromise sterility of the product

To maintain sterility of nearby sterile materials, a proper aseptic manipulation should be approached from the side and not above the product (in vertical unidirectional flow operations). Also, operators should refrain from speaking when in direct proximity to the critical area.

- Maintain Proper Gown Control

Prior to and throughout aseptic operations, an operator should not engage in any activity that poses an unreasonable contamination risk to the gown.

Only personnel who are qualified and appropriately gowned should be permitted access to the aseptic manufacturing area. The gown should provide a barrier between the body and exposed sterilized materials and prevent contamination from particles generated by, and microorganisms shed from, the body. The Agency recommends gowns that are sterilized and nonshedding, and cover the skin and hair (face-masks, hoods, beard/moustache covers, protective goggles, and elastic gloves are examples of common elements of gowns). Written procedures should detail the methods used to don each gown component in an aseptic manner. An adequate barrier should be created by the overlapping of gown components (e.g., gloves overlapping sleeves). If an element of a gown is found to be torn or defective, it should be changed immediately. Gloves should be sanitized frequently.

There should be an established program to regularly assess or audit conformance of personnel to relevant aseptic manufacturing requirements. An aseptic gowning qualification program should assess the ability of a cleanroom operator to maintain the quality of the gown after performance of gowning procedures. We recommend that this assessment include microbiological surface sampling of several locations on a gown (e.g., glove fingers, facemask, forearm, chest). Sampling sites should be justified. Following an initial assessment of gowning, periodic requalification will provide for the monitoring of various gowning locations over a suitable period to ensure consistent acceptability of aseptic gowning techniques. Annual requalification is normally sufficient for those automated operations where personnel involvement is minimized and monitoring data indicate environmental control. For any aseptic processing operation, if adverse conditions occur, additional or more frequent requalification could be indicated.

To protect exposed sterilized product, personnel should to maintain gown quality and strictly adhere to appropriate aseptic techniques. Written procedures should adequately address circumstances under which personnel should be retrained, requalified, or reassigned to other areas.

B.   Laboratory Personnel

The basic principles of training, aseptic technique, and personnel qualification in aseptic manufacturing also are applicable to those performing aseptic sampling and microbiological laboratory analyses. Processes and systems cannot be considered to be in control and reproducible if the validity of data produced by the laboratory is in question.

C.   Monitoring Program

Personnel can significantly affect the quality of the environment in which the sterile product is processed. A vigilant and responsive personnel monitoring program should be established. Monitoring should be accomplished by obtaining surface samples of each operator's gloves on a daily basis, or in association with each lot. This sampling should be accompanied by an appropriate sampling frequency for other strategically selected locations of the gown (Ref. 5). The quality control unit should establish a more comprehensive monitoring program for operators involved in operations which are especially labor intensive (i.e., those requiring repeated or complex aseptic manipulations).

Asepsis is fundamental to an aseptic processing operation. An ongoing goal for manufacturing personnel in the aseptic processing room is to maintain contamination-free gloves and gowns throughout operations. Sanitizing gloves just prior to sampling is inappropriate because it can prevent recovery of microorganisms that were present during an aseptic manipulation. When operators exceed established levels or show an adverse trend, an investigation should be conducted promptly. Follow-up actions can include increased sampling, increased observation, retraining, gowning requalification, and in certain instances, reassignment of the individual to operations outside of the aseptic manufacturing area. Microbiological trending systems, and assessment of the impact of atypical trends, are discussed in more detail under Section X. Laboratory Controls.

VI. COMPONENTS AND CONTAINER/CLOSURES

21 CFR 210.3(b)(3) states that "*Component* means any ingredient intended for use in the manufacture of a drug product, including those that may not appear in such drug product."

21 CFR 211.80(a) states that "There shall be written procedures describing in sufficient detail the receipt, identification, storage, handling, sampling, testing, and approval or rejection of components and drug product containers and closures; such written procedures shall be followed."

21 CFR 211.80(b) states that "Components and drug product containers and closures shall at all times be handled and stored in a manner to prevent contamination."

21 CFR 211.84(d) states, in part, that "Samples shall be examined and tested as follows: * * * (6) Each lot of a component, drug product container, or closure that is liable to microbiological contamination that is objectionable in view of its intended use shall be subjected to microbiological tests before use."

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21 CFR 211.94(c) states that "Drug product containers and closures shall be clean and, where indicated by the nature of the drug, sterilized and processed to remove pyrogenic properties to assure that they are suitable for their intended use."

21 CFR 211.94(d) states that "Standards or specifications, methods of testing, and, where indicated, methods of cleaning, sterilizing, and processing to remove pyrogenic properties shall be written and followed for drug product containers and closures."

21 CFR 211.113(b) states that "Appropriate written procedures, designed to prevent microbiological contamination of drug products purporting to be sterile, shall be established and followed. Such procedures shall include validation of any sterilization process."

A.   Components

A drug product produced by aseptic processing can become contaminated through the use of one or more components that are contaminated with microorganisms or endotoxins. Examples of components include active ingredients, Water for Injection (WFI), and other excipients. It is important to characterize the microbial content (e.g., bioburden, endotoxin) of each component that could be contaminated and establish appropriate acceptance limits.

Endotoxin load data are significant because parenteral products are intended to be nonpyrogenic. There should be written procedures and appropriate specifications for acceptance or rejection of each lot of components that might contain endotoxins. Any components failing to meet defined endotoxin limits should be rejected.

In aseptic processing, each component is individually sterilized or several components are combined, with the resulting mixture sterilized.[11] Knowledge of bioburden is important in assessing whether a sterilization process is adequate. Several methods can be suitable for sterilizing components (see relevant discussion in Section IX). A widely used method is filtration of a solution formed by dissolving the component(s) in a solvent such as Water For Injection, USP. The solution is passed through a sterilizing membrane or cartridge filter. Filter sterilization is used where the component is soluble and is likely to be adversely affected by heat. A variation of this method includes subjecting the filtered solution to aseptic crystallization and precipitation (or lyophilization) of the component as a sterile powder. However, this method involves more handling and manipulation and therefore has a higher potential for contamination during processing.

Dry heat sterilization is a suitable method for components that are heat stable and insoluble. However, conducting carefully designed heat penetration and distribution studies is of particular significance for powder sterilization because of the insulating effects of the powder.

Irradiation can be used to sterilize some components. Studies should be conducted to demonstrate that the process is appropriate for the component.

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[11] See Appendix III for discussion of certain biologic components that are aseptically handled from the start of the process.

B. Containers/Closures

1. Preparation

Containers and closures should be rendered sterile and, for parenteral drug products, nonpyrogenic. The process used will depend primarily on the nature of the container and/or closure materials. The validation study for such a process should be adequate to demonstrate its ability to render materials sterile and non-pyrogenic. Written procedures should specify the frequency of revalidation of these processes as well as time limits for holding sterile, depyrogenated containers and closures.

Pre-sterilization preparation of glass containers usually involves a series of wash and rinse cycles. These cycles serve an important role in removing foreign matter. We recommend use of rinse water of high purity so as not to contaminate containers. For parenteral products, final rinse water should meet the specifications of WFI, USP.

The adequacy of the depyrogenation process can be assessed by spiking containers and closures with known quantities of endotoxin, followed by measuring endotoxin content after depyrogenation. The challenge studies can generally be performed by directly applying a reconstituted endotoxin solution onto the surfaces being tested. The endotoxin solution should then be allowed to air dry. Positive controls should be used to measure the percentage of endotoxin recovery by the test method. Validation study data should demonstrate that the process reduces the endotoxin content by at least 99.9 percent (3 logs) (see Section VII).[12]

Subjecting glass containers to dry heat generally accomplishes both sterilization and depyrogenation. Validation of dry heat sterilization and depyrogenation should include appropriate heat distribution and penetration studies as well as the use of worst-case process cycles, container characteristics (e.g., mass), and specific loading configurations to represent actual production runs. See Section IX.C. Plastic containers used for parenteral products also should be non-pyrogenic. Where applicable, multiple WFI rinses can be effective in removing pyrogens from these containers.

Plastic containers can be sterilized with an appropriate gas, irradiation, or other suitable means. For gases such as Ethylene Oxide (EtO), certain issues should receive attention. For example, the parameters and limits of the EtO sterilization cycle (e.g., temperature, pressure, humidity, gas concentration, exposure time, degassing, aeration, and determination of residuals) should be specified and monitored closely. EtO is an effective surface sterilant and is also used to penetrate certain packages with porous overwrapping. Biological indicators are of special importance in demonstrating the effectiveness of EtO and other gas sterilization processes. We recommend that these methods be carefully controlled and validated to evaluate whether consistent penetration of the sterilant can be achieved and to minimize residuals. Residuals from EtO processes typically include ethylene oxide as well as its byproducts, and should be within specified limits.

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[12] When this level of depyrogenation by dry heat has been successfully validated using endotoxin challenge, a sterilization validation using a biological indicator challenge would not be indicated.

Rubber closures (e.g., stoppers and syringe plungers) can be cleaned by multiple cycles of washing and rinsing prior to final steam or irradiation sterilization. At minimum, the initial rinses for the washing process should employ at least Purified Water, USP, of minimal endotoxin content, followed by final rinse(s) with WFI for parenteral products. Normally, depyrogenation can be achieved by multiple rinses of hot WFI. The time between washing, drying (where appropriate), and sterilizing should be minimized because residual moisture on the stoppers can support microbial growth and the generation of endotoxins. Because rubber is a poor conductor of heat, extra attention is indicated in the validation of processes that use heat with respect to its penetration into the rubber stopper load (See Section IX.C). Validation data from the washing procedure should demonstrate successful endotoxin removal from rubber materials.

A potential source of contamination is the siliconization of rubber stoppers. Silicone used in the preparation of rubber stoppers should meet appropriate quality control criteria and not have an adverse effect on the safety, quality, or purity of the drug product.

Contract facilities that perform sterilization and/or depyrogenation of containers and closures are subject to the same CGMP requirements as those established for in-house processing. The finished dosage form manufacturer should review and assess the contractor's validation protocol and final validation report. In accord with 211.84(d)(3), a manufacturer who establishes the reliability of the supplier's test results at appropriate intervals may accept containers or closures based on visual identification and Certificate of Analysis review.

2. Inspection of Container Closure System

A container closure system that permits penetration of microorganisms is unsuitable for a sterile product. Any damaged or defective units should be detected, and removed, during inspection of the final sealed product. Safeguards should be implemented to strictly preclude shipment of product that may lack container closure integrity and lead to nonsterility. Equipment suitability problems or incoming container or closure deficiencies can cause loss of container closure system integrity. For example, failure to detect vials fractured by faulty machinery as well as by mishandling of bulk finished stock has led to drug recalls. If damage that is not readily detected leads to loss of container closure integrity, improved procedures should be rapidly implemented to prevent and detect such defects.

Functional defects in delivery devices (e.g., syringe device defects, delivery volume) can also result in product quality problems and should be monitored by appropriate in-process testing.

Any defects or results outside the specifications established for in-process and final inspection are to be investigated in accord with § 211.192.

VII. ENDOTOXIN CONTROL

21 CFR 211.63 states that "Equipment used in the manufacture, processing, packing, or holding of a drug product shall be of appropriate design, adequate size, and suitably located to facilitate operations for its intended use and for its cleaning and maintenance."

21 CFR 211.65(a) states that "Equipment shall be constructed so that surfaces that contact components, in-process materials, or drug products shall not be reactive, additive, or absorptive so as to alter the safety, identity, strength, quality, or purity of the drug product beyond the official or other established requirements."

21 CFR 211.67(a) states that "Equipment and utensils shall be cleaned, maintained, and sanitized at appropriate intervals to prevent malfunctions or contamination that would alter the safety, identify, strength, quality, or purity of the drug product beyond the official or other established requirements."

21 CFR 211.94(c) states that "Drug product containers and closures shall be clean and, where indicated by the nature of the drug, sterilized and processed to remove pyrogenic properties to assure that they are suitable for their intended use."

21 CFR 211.167(a) states that "For each batch of drug product purporting to be sterile and/or pyrogen-free, there shall be appropriate laboratory testing to determine conformance to such requirements. The test procedures shall be in writing and shall be followed."

Endotoxin contamination of an injectable product can occur as a result of poor CGMP controls. Certain patient populations (e.g., neonates), those receiving other injections concomitantly, or those administered a parenteral in atypically large volumes or doses can be at greater risk for pyrogenic reaction than anticipated by the established limits based on body weight of a normal healthy adult (Ref. 6, 7). Such clinical concerns reinforce the importance of exercising appropriate CGMP controls to prevent generation of endotoxins. Drug product components, containers, closures, storage time limitations, and manufacturing equipment are among the areas to address in establishing endotoxin control.

Adequate cleaning, drying, and storage of equipment will control bioburden and prevent contribution of endotoxin load. Equipment should be designed to be easily assembled and disassembled, cleaned, sanitized, and/or sterilized. If adequate procedures are not employed, endotoxins can be contributed by both upstream and downstream processing equipment.

Sterilizing-grade filters and moist heat sterilization have not been shown to be effective in removing endotoxin. Endotoxin on equipment surfaces can be inactivated by high-temperature dry heat, or removed from equipment surfaces by cleaning procedures. Some clean-in-place procedures employ initial rinses with appropriate high purity water and/or a cleaning agent (e.g., acid, base, surfactant), followed by final rinses with heated WFI. Equipment should be dried following cleaning, unless the equipment proceeds immediately to the sterilization step.

VIII. TIME LIMITATIONS

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21 CFR 211.111 states that "When appropriate, time limits for the completion of each phase of production shall be established to assure the quality of the drug product. Deviation from established time limits may be acceptable if such deviation does not compromise the quality of the drug product. Such deviation shall be justified and documented."

When appropriate, time limits must be established for each phase of aseptic processing (§ 211.111). Time limits should include, for example, the period between the start of bulk product compounding and its sterilization, filtration processes, product exposure while on the processing line, and storage of sterilized equipment, containers and closures. The time limits established for the various production phases should be supported by data. Bioburden and endotoxin load should be assessed when establishing time limits for stages such as the formulation processing stage.

The total time for product filtration should be limited to an established maximum to prevent microorganisms from penetrating the filter. Such a time limit should also prevent a significant increase in upstream bioburden and endotoxin load. Because they can provide a substrate for microbial attachment, maximum use times for those filters used upstream for solution clarification or particle removal should also be established and justified.

IX. VALIDATION OF ASEPTIC PROCESSING AND STERILIZATION

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21 CFR 211.63, 211.65, and 211.67 address, respectively, "Equipment design, size, and location," "Equipment construction," and "Equipment cleaning and maintenance."

21 CFR 211.84(c) states, in part, that "Samples shall be collected in accordance with the following procedures: * * * (3) Sterile equipment and aseptic sampling techniques shall be used when necessary."

21 CFR 211.100(a) states, in part, that "There shall be written procedures for production and process control designed to assure that the drug products have the identity, strength, quality, and purity they purport or are represented to possess. Such procedures shall include all requirements in this subpart * * *."

21 CFR 211.113(b) states that "Appropriate written procedures, designed to prevent microbiological contamination of drug products purporting to be sterile, shall be established and followed. Such procedures shall include validation of any sterilization process."

This section primarily discusses routine qualification and validation study recommendations. Change control procedures are addressed only briefly, but are an important part of the quality systems established by a firm. A change in facility, equipment, process, or test method should be evaluated through the written change control program, triggering an evaluation of the need for revalidation or requalification.

A. Process Simulations

To ensure the sterility of products purporting to be sterile, sterilization, aseptic filling and closing operations must be adequately validated (§ 211.113). The goal of even the most effective sterilization processes can be defeated if the sterilized elements of a product (the drug formulation, the container, and the closure) are brought together under conditions that contaminate any of those elements.

An aseptic processing operation should be validated using a microbiological growth medium in place of the product. This *process simulation,* also known as a *media fill,* normally includes exposing the microbiological growth medium to product contact surfaces of equipment, container closure systems, critical environments, and process manipulations to closely simulate the same exposure that the product itself will undergo. The sealed containers filled with the medium are then incubated to detect microbial contamination. Results are then interpreted to assess the potential for a unit of drug product to become contaminated during actual operations (e.g., start-up, sterile ingredient additions, aseptic connections, filling, closing). Environmental monitoring data from the process simulation can also provide useful information for the processing line evaluation.

1. Study Design

A media fill program should incorporate the contamination risk factors that occur on a production line, and accurately assesses the state of process control. Media fill studies should closely simulate aseptic manufacturing operations incorporating, as appropriate, worst-case activities and conditions that provide a challenge to aseptic operations. FDA recommends that the media fill program address applicable issues such as:

- Factors associated with the longest permitted run on the processing line that can pose contamination risk (e.g., operator fatigue)

- Representative number, type, and complexity of normal interventions that occur with each run, as well as nonroutine interventions and events (e.g., maintenance, stoppages, equipment adjustments)

- Lyophilization, when applicable

- Aseptic assembly of equipment (e.g., at start-up, during processing)

- Number of personnel and their activities

- Representative number of aseptic additions (e.g., charging containers and closures as well as sterile ingredients) or transfers

- Shift changes, breaks, and gown changes (when applicable)

- Type of aseptic equipment disconnections/connections

- Aseptic sample collections

- Line speed and configuration

- Weight checks

- Container closure systems (e.g., sizes, type, compatibility with equipment)

- Specific provisions in written procedures relating to aseptic processing (e.g., conditions permitted before line clearance is mandated)

A written batch record, documenting production conditions and simulated activities, should be prepared for each media fill run. The same vigilance should be observed in both media fill and routine production runs. The firm's rationale for the conditions and activities simulated during the media fill should be clearly defined. Media fills should not be used to justify practices that pose unnecessary contamination risks.

2. Frequency and Number of Runs

When a processing line is initially qualified, individual media fills should be repeated enough times to ensure that results are consistent and meaningful. This approach is important because a single run can be inconclusive, while multiple runs with divergent results signal a process that is not in control. We recommend that at least three consecutive separate successful runs be performed during initial line qualification. Subsequently, routine semi-annual qualification conducted for each processing line will evaluate the state of control of the aseptic process. Activities and interventions representative of each shift, and shift changeover, should be incorporated into the design of the semi-annual qualification program. For example, the evaluation of a production shift should address its unique time-related and operational features.[13] All personnel who are authorized to enter the aseptic processing room during manufacturing, including technicians and maintenance personnel, should participate in a media fill at least once a year. Participation should be consistent with the nature of each operator's duties during routine production.

Each change to a product or line change should be evaluated using a written change control system. Any changes or events that have the potential to affect the ability of the aseptic process to exclude contamination from the sterilized product should be assessed through additional media fills. For example, facility and equipment modifications, line configuration changes, significant changes in personnel, anomalies in environmental testing results, container closure system changes, extended shutdowns, or end product sterility testing showing contaminated products may be cause for revalidation of the system.

When data from a media fill indicate the process may not be in control, an investigation should be conducted to determine the origin of the contamination and the scope of the problem. Once corrections are instituted, process simulation run(s) should be performed to confirm that deficiencies have been corrected and the process has returned to a state of control. When an investigation fails to reach well-supported, substantive conclusions as to the cause of the media fill failure, three consecutive successful runs in tandem with increased scrutiny of the production process may be warranted.

3. Duration of Runs

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[13] One example might be the movement of personnel into and out of the aseptic processing and gowning change rooms during a shift change.

The duration of aseptic processing operations is a major consideration in media fill design. Although the most accurate simulation model would be the full batch size and duration because it most closely simulates the actual production operations, other appropriate models can be justified. The duration of the media fill run should be determined by the time it takes to incorporate manipulations and interventions, as well as appropriate consideration of the duration of the actual aseptic processing operation. Interventions that commonly occur should be routinely simulated, while those occurring rarely can be simulated periodically.

While conventional manufacturing lines are usually automated, operated at relatively high speeds, and designed to limit operator intervention, some processes still include considerable operator involvement. When aseptic processing employs manual filling or closing, or extensive manual manipulations, the duration of the process simulation should generally be no less than the length of the actual manufacturing process to best simulate contamination risks posed by operators.

For lyophilization operations, FDA recommends that unsealed containers be exposed to partial evacuation of the chamber in a manner that simulates the process. Vials should not be frozen, and precautions should be taken that ensure that the medium remains in an aerobic state to avoid potentially inhibiting the growth of microorganisms.

4. Size of Runs

The simulation run sizes should be adequate to mimic commercial production conditions and accurately assess the potential for commercial batch contamination. The number of units filled during the process simulation should be based on contamination risk for a given process and sufficient to accurately simulate activities that are representative of the manufacturing process. A generally acceptable starting point for run size is in the range of 5,000 to 10,000 units. For operations with production sizes under 5,000, the number of media filled units should at least equal the maximum batch size made on the processing line (Ref. 8).

When the possibility of contamination is higher based on the process design (e.g., manually intensive filling lines), a larger number of units, generally at or approaching the full production batch size, should be used. In contrast, a process conducted in an isolator (see Appendix 1) can have a low risk of contamination because of the lack of direct human intervention and can be simulated with a lower number of units as a proportion of the overall operation.

Media fill size is an especially important consideration because some batches are produced over multiple shifts or yield an unusually large number of units. These factors should be carefully evaluated when designing the simulation to adequately encompass conditions and any potential risks associated with the larger operation.

5. Line Speed

The media fill program should adequately address the range of line speeds employed during production. Each media fill run should evaluate a single line speed, and the speed chosen should be justified. For example, use of high line speed is often most appropriate in the evaluation of manufacturing processes characterized by frequent interventions or a significant degree of

manual manipulation. Use of slow line speed is generally appropriate for evaluating manufacturing processes with prolonged exposure of the sterile drug product and containers/closures in the aseptic area.

6. Environmental Conditions

Media fills should be adequately representative of the conditions under which actual manufacturing operations are conducted. An inaccurate assessment (making the process appear cleaner than it actually is) can result from conducting a media fill under extraordinary air particulate and microbial quality, or under production controls and precautions taken in preparation for the media fill. To the extent standard operating procedures permit stressful conditions (e.g., maximum number of personnel present and elevated activity level), it is important that media fills include analogous challenges to support the validity of these studies. Stressful conditions do not include artificially created environmental extremes, such as reconfiguration of HVAC systems to operate at worst-case limits.

7. Media

In general, a microbiological growth medium, such as soybean casein digest medium, should be used. Use of anaerobic growth media (e.g., fluid thioglycollate medium) should be considered in special circumstances. The media selected should be demonstrated to promote growth of gram-positive and gram-negative bacteria, and yeast and mold (e.g., USP indicator organisms). The QC laboratory should determine if USP indicator organisms sufficiently represent production-related isolates. Environmental monitoring and sterility test isolates can be substituted (as appropriate) or added to the growth promotion challenge. Growth promotion units should be inoculated with a <100 CFU challenge. If the growth promotion testing fails, the origin of any contamination found during the simulation should nonetheless be investigated and the media fill promptly repeated.[14]

The production process should be accurately simulated using media and conditions that optimize detection of any microbiological contamination. Each unit should be filled with an appropriate quantity and type of microbial growth medium to contact the inner container closure surfaces (when the unit is inverted or thoroughly swirled) and permit visual detection of microbial growth.

Some drug manufacturers have expressed concern over the possible contamination of the facility and equipment with nutrient media during media fill runs. However, if the medium is handled properly and is promptly followed by the cleaning, sanitizing, and, where necessary, sterilization of equipment, subsequently processed products are not likely to be compromised.

8. Incubation and Examination of Media-Filled Units

Media units should be incubated under conditions adequate to detect microorganisms that might otherwise be difficult to culture. Incubation conditions should be established in accord with the following general guidelines:

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[14] The cause of the growth promotion failure should also be investigated.

- Incubation temperature should be suitable for recovery of bioburden and environmental isolates and should at no time be outside the range of 20-35°C. Incubation temperature should be maintained within ±2.5°C of the target temperature.

- Incubation time should not be less than 14 days. If two temperatures are used for the incubation of the media filled units, the units should be incubated for at least 7 days at each temperature (starting with the lower temperature).

Each media-filled unit should be examined for contamination by personnel with appropriate education, training, and experience in inspecting media fill units for microbiological contamination. If QC personnel do not perform the inspection, there should be QC unit oversight throughout any such examination. All suspect units identified during the examination should be brought to the immediate attention of the QC microbiologist. To allow for visual detection of microbial growth, we recommend substituting clear containers (with otherwise identical physical properties) for amber or other opaque containers. If appropriate, other methods can also be considered to ensure visual detection.

When a firm performs a final product inspection of units immediately following the media fill run, all integral units should proceed to incubation. Units found to have defects not related to integrity (e.g., cosmetic defect) should be incubated; units that lack integrity should be rejected. Erroneously rejected units should be returned promptly for incubation with the media fill lot.

After incubation is underway, any unit found to be damaged should be included in the data for the media fill run, because the units can be representative of drug product released to the market. Any decision to exclude such incubated units (i.e., non-integral) from the final run tally should be fully justified and the deviation explained in the media fill report. If a correlation emerges between difficult to detect damage and microbial contamination, a thorough investigation should be conducted to determine its cause (see Section VI.B).

Written procedures regarding aseptic interventions should be clear and specific (e.g., intervention type; quantity of units removed), providing for consistent production practices and assessment of these practices during media fills. If written procedures and batch documentation are adequate to describe an associated clearance, the intervention units removed during media fills do not need to be incubated.[15] Where procedures lack specificity, there would be insufficient justification for exclusion of units removed during an intervention from incubation. For example, if a production procedure requires removal of 10 units after an intervention at the stoppering station infeed, batch records (i.e., for production and media fills) should clearly document conformance with this procedure. In no case should more units be removed during a media fill intervention than would be cleared during a production run.

The ability of a media fill run to detect potential contamination from a given simulated activity should not be compromised by a large-scale line clearance. We recommend incorporating

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[15] To assess contamination risks during initial aseptic setup (before fill), valuable information can be obtained by incubating all such units that may be normally removed. These units are typically incubated separately, and would not necessarily be included in the acceptance criteria for the media fill.

appropriate study provisions to avoid and address a large line clearance that results in the removal of a unit possibly contaminated during an unrelated event or intervention.

Appropriate criteria should be established for yield[16] and accountability (reconciliation of filled units). Media fill record reconciliation documentation should include a full accounting and description of units rejected from a batch.

9.   Interpretation of Test Results

The process simulation run should be observed by the QC Unit, and contaminated units should be reconcilable with the approximate time and the activity being simulated during the media fill. Video recording of a media fill may serve as a useful aide in identifying personnel practices that could negatively affect the aseptic process.

Any contaminated unit should be considered objectionable and investigated. The microorganisms should be identified to species level. The investigation should survey the possible causes of contamination. In addition, any failure investigation should assess the impact on commercial drugs produced on the line since the last media fill.

Whenever contamination exists in a media fill run, it should be considered indicative of a potential sterility assurance problem, regardless of run size. The number of contaminated units should not be expected to increase in a directly proportional manner with the number of vials in the media fill run. Test results should reliably and reproducibly show that the units produced by an aseptic processing operation are sterile. Modern aseptic processing operations in suitably designed facilities have demonstrated a capability of meeting contamination levels approaching zero (Ref. 8, 9) and should normally yield no media fill contamination. Recommended criteria for assessing state of aseptic line control are as follows:

- When filling fewer than 5000 units, no contaminated units should be detected.
  -- One (1) contaminated unit is considered cause for revalidation, following an investigation.

- When filling from 5,000 to 10,000 units:
  -- One (1) contaminated unit should result in an investigation, including consideration of a repeat media fill.
  -- Two (2) contaminated units are considered cause for revalidation, following investigation.

- When filling more than 10,000 units:
  -- One (1) contaminated unit should result in an investigation.
  -- Two (2) contaminated units are considered cause for revalidation, following investigation.

For any run size, intermittent incidents of microbial contamination in media filled runs can be indicative of a persistent low-level contamination problem that should be investigated.

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[16]Total units incubated/total number of units filled.

Accordingly, recurring incidents of contaminated units in media fills for an individual line, regardless of acceptance criteria, would be a signal of an adverse trend on the aseptic processing line that should lead to problem identification, correction, and revalidation.

A firm's use of media fill acceptance criteria allowing infrequent contamination does not mean that a distributed lot of drug product purporting to be sterile may contain a nonsterile unit. The purpose of an aseptic process is to prevent any contamination. A manufacturer is fully liable for the shipment of any nonsterile unit, an act that is prohibited under the FD&C Act (Section 301(a) 21 U.S.C. 331(a)). FDA also recognizes that there might be some scientific and technical limitations on how precisely and accurately process simulations can characterize a system of controls intended to exclude contamination.

As with any process validation run, it is important to note that *invalidation* of a media fill run should be a rare occurrence. A media fill run should be aborted only under circumstances in which written procedures require commercial lots to be equally handled. Supporting documentation and justification should be provided in such cases.

B.   Filtration Efficacy

Filtration is a common method of sterilizing drug product solutions. A sterilizing grade filter should be validated to reproducibly remove viable microorganisms from the process stream, producing a sterile effluent.[17] Currently, such filters usually have a rated pore size of 0.2 µm or smaller.[18] Use of redundant sterilizing filters should be considered in many cases. Whatever filter or combination of filters is used, validation should include microbiological challenges to simulate worst-case production conditions for the material to be filtered and integrity test results of the filters used for the study. Product bioburden should be evaluated when selecting a suitable challenge microorganism to assess which microorganism represents the worst-case challenge to the filter. The microorganism *Brevundimonas diminuta* (ATCC 19146) when properly grown, harvested and used, is a common challenge microorganism for 0.2 µm rated filters because of its small size (0.3 µm mean diameter). The manufacturing process controls should be designed to minimize the bioburden of the unfiltered product. Bioburden of unsterilized bulk solutions should be determined to trend the characteristics of potentially contaminating organisms.

In certain cases, when justified as equivalent or better than use of *B. diminuta*, it may be appropriate to conduct bacterial retention studies with a bioburden isolate. The number of microorganisms in the challenge is important because a filter can contain a number of pores larger than the nominal rating, which has the potential to allow passage of microorganisms. The probability of such passage is considered to increase as the number of organisms (bioburden) in the material to be filtered increases. A challenge concentration of at least 10^7 organisms per cm^2 of effective filtration area should generally be used, resulting in no passage of the challenge microorganism. The challenge concentration used for validation is intended to provide a margin of safety well beyond what would be expected in production.

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[17] This document does not address virus removal.

[18] 0.22µ and 0.2µ are considered interchangeable nominal pore size ratings.

Direct inoculation into the drug formulation is the preferred method because it provides an assessment of the effect of drug product on the filter matrix and on the challenge organism. However, directly inoculating *B. diminuta* into products with inherent bactericidal activity against this microbe, or into oil-based formulations, can lead to erroneous conclusions. When sufficiently justified, the effects of the product formulation on the membrane's integrity can be assessed using an appropriate alternate method. For example, a drug product could be filtered in a manner in which the worst-case combination of process specifications and conditions are simulated. This step could be followed by filtration of the challenge organism for a significant period of time, under the same conditions, using an appropriately modified product (e.g., lacking an antimicrobial preservative or other antimicrobial component) as the vehicle. Any divergence from a simulation using the actual product and conditions of processing should be justified.

Factors that can affect filter performance generally include (1) viscosity and surface tension of the material to be filtered, (2) pH, (3) compatibility of the material or formulation components with the filter itself, (4) pressures, (5) flow rates, (6) maximum use time, (7) temperature, (8) osmolality, (9) and the effects of hydraulic shock. When designing the validation protocol, it is important to address the effect of the extremes of processing factors on the filter capability to produce sterile effluent. Filter validation should be conducted using the worst-case conditions, such as maximum filter use time and pressure (Ref. 12). Filter validation experiments, including microbial challenges, need not be conducted in the actual manufacturing areas. However, it is essential that laboratory experiments simulate actual production conditions. The specific type of filter membrane used in commercial production should be evaluated in filter validation studies. There are advantages to using production filters in these bacterial retention validation studies. When the more complex filter validation tests go beyond the capabilities of the filter user, tests are often conducted by outside laboratories or by filter manufacturers. However, it is the responsibility of the filter user to review the validation data on the efficacy of the filter in producing a sterile effluent. The data should be applicable to the user's products and conditions of use because filter performance may differ significantly for various conditions and products.

After a filtration process is properly validated for a given product, process, and filter, it is important to ensure that identical filters (e.g., of identical polymer construction and pore size rating) are used in production runs. Sterilizing filters should be routinely discarded after processing of a single lot. However, in those instances when repeated use can be justified, the sterile filter validation should incorporate the maximum number of lots to be processed. Integrity testing of the filter(s) can be performed prior to processing, and should be routinely performed post-use. It is important that integrity testing be conducted after filtration to detect any filter leaks or perforations that might have occurred during the filtration. *Forward flow and bubble point* tests, when appropriately employed, are two integrity tests that can be used. A production filter's integrity test specification should be consistent with data generated during bacterial retention validation studies.

C.   Sterilization of Equipment, Containers, and Closures

Equipment surfaces that contact sterilized drug product or its sterilized containers or closures must be sterile so as not to alter purity of the drug (211.67 and 211.113). Where reasonable contamination potential exists, surfaces that are in the vicinity of the sterile product should also be rendered free of viable organisms. It is as important in aseptic processing to validate the

processes used to sterilize such critical equipment as it is to validate processes used to sterilize the drug product and its container and closure. Moist heat and dry heat sterilization, the most widely used, are the primary processes discussed in this document. However, many of the heat sterilization principles discussed in this guidance are also applicable to other sterilization methods.

Sterility of aseptic processing equipment should normally be maintained by sterilization between each batch.[19] Following sterilization, transportation and assembly of equipment, containers, and closures should be performed with strict adherence to aseptic methods in a manner that protects and sustains the product's sterile state.

1.   Qualification and Validation

Validation studies should be conducted to demonstrate the efficacy of the sterilization cycle. Requalification studies should also be performed on a periodic basis. The specific load configurations, as well as biological indicator and temperature sensor locations, should be documented in validation records. Batch production records should subsequently document adherence to the validated load patterns.

It is important to remove air from the autoclave chamber as part of a steam sterilization cycle. The insulating properties of air interfere with the ability of steam to transfer its energy to the load, achieving lower lethality than associated with saturated steam. It also should be noted that the resistance of microorganisms can vary widely depending on the material to be sterilized. For this reason, careful consideration should be given during sterilization validation to the nature or type of material chosen as the carrier of the biological indicator to ensure an appropriately representative study.

Potentially difficult to reach locations within the sterilizer load or equipment train (for SIP applications) should be evaluated. For example, filter installations in piping can cause a substantial pressure differential across the filter, resulting in a significant temperature drop on the downstream side. We recommend placing biological indicators at appropriate downstream locations of the filter.

Empty chamber studies evaluate numerous locations throughout a sterilizing unit (e.g., steam autoclave, dry heat oven) or equipment train (e.g., large tanks, immobile piping) to confirm uniformity of conditions (e.g., temperature, pressure). These uniformity or *mapping* studies should be conducted with calibrated measurement devices.

Heat penetration studies should be performed using the established sterilizer loads. Validation of the sterilization process with a loaded chamber demonstrates the effects of loading on thermal input to the items being sterilized and may identify difficult to heat or penetrate items where there could be insufficient lethality to attain sterility. The placement of biological indicators at numerous positions in the load, including the most difficult to sterilize places, is a direct means of confirming the efficacy of any sterilization procedure. In general, the biological indicator should be placed adjacent to the temperature sensor so as to assess the correlation between microbial lethality and predicted lethality based on thermal input. When determining which

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[19] If appropriate, alternate intervals can be defined, justified, and supported by validation studies.

articles are difficult to sterilize, special attention should be given to the sterilization of filters, filling manifolds, and pumps. Some other examples include certain locations of tightly wrapped or densely packed supplies, securely fastened load articles, lengthy tubing, the sterile filter apparatus, hydrophobic filters, and stopper load.

Ultimately, cycle specifications for such sterilization methods should be based on the delivery of adequate lethality to the slowest to heat locations. A sterility assurance level of 10^{-6} or better should be demonstrated for a sterilization process. For more information, please also refer to the FDA guidance entitled *Guideline for the Submission of Documentation for Sterilization Process Validation in Applications for Human and Veterinary Drug Products.*

The sterilizer validation program should continue to focus on the load areas identified as most difficult to penetrate or heat. The suitability of the sterilizer should be established by qualification, maintenance, change control, and periodic verification of the cycle, including biological challenges. Change control procedures should adequately address issues such as a load configuration change or a modification of a sterilizer.

> 2. *Equipment Controls and Instrument Calibration*

For both validation and routine process control, the reliability of the data generated by sterilization cycle monitoring devices should be considered to be of the utmost importance. Devices that measure cycle parameters should be routinely calibrated. Written procedures should be established to ensure that these devices are maintained in a calibrated state. For example, we recommend that procedures address the following:

- Temperature and pressure monitoring devices for heat sterilization should be calibrated at suitable intervals. The sensing devices used for validation studies should be calibrated before and after validation runs.
- Devices used to monitor dwell time in the sterilizer should be periodically calibrated.
- The microbial count of a biological indicator should be confirmed. Biological indicators should be stored under appropriate conditions.
- If the reliability of a vendor's Certificate of Analysis is established through an appropriate qualification program, the D-value of a biological indicator (e.g., spore strips, glass ampuls) can be accepted in lieu of confirmatory testing of each lot. However, a determination of resistance (D-value) should be performed for any biological indicator inoculated onto a substrate, or used in a way that is other than described by the vendor. D-value determinations can be conducted by an independent laboratory.
- Where applicable, instruments used to determine the purity of steam should be calibrated.
- For dry heat depyrogenation tunnels, devices (e.g. sensors and transmitters) used to measure belt speed should be routinely calibrated. Bacterial endotoxin challenges should be appropriately prepared and measured by the laboratory.

To ensure robust process control, equipment should be properly designed with attention to features such as accessibility to sterilant, piping slope, and proper condensate removal (as applicable). Equipment control should be ensured through placement of measuring devices at

those control points that are most likely to rapidly detect unexpected process variability. Where manual manipulations of valves are required for sterilizer or SIP operations, these steps should be documented in manufacturing procedures and batch records. Sterilizing equipment should be properly maintained to allow for consistent, satisfactory function. Routine evaluation of sterilizer performance-indicating attributes, such as equilibrium (come up) time is important in assuring that the unit continues to operate as per the validated conditions.

X.   LABORATORY CONTROLS

21 CFR 211.22(b) states that "Adequate laboratory facilities for the testing and approval (or rejection) of components, drug product containers, closures, packaging materials, in-process materials, and drug products shall be available to the quality control unit."

21 CFR 211.22(c) states that "The quality control unit shall have the responsibility for approving or rejecting all procedures or specifications impacting on the identity, strength, quality, and purity of the drug product."

21 CFR 211.42(c) states, in part, that "Operations shall be performed within specifically defined areas of adequate size. There shall be separate or defined areas or such other control systems for the firm's operations as are necessary to prevent contamination or mixups during the course of the following procedures: * * * (10) Aseptic processing, which includes as appropriate: * * * (iv) A system for monitoring environmental conditions; * * *."

21 CFR 211.56(b) states that "There shall be written procedures assigning responsibility for sanitation and describing in sufficient detail the cleaning schedules, methods, equipment, and materials to be used in cleaning the buildings and facilities; such written procedures shall be followed."

21 CFR 211.56(c) states, in part, that "There shall be written procedures for use of suitable rodenticides, insecticides, fungicides, fumigating agents, and cleaning and sanitizing agents. Such written procedures shall be designed to prevent the contamination of equipment, components, drug product containers, closures, packaging, labeling materials, or drug products and shall be followed * * *."

21 CFR 211.110(a) states, in part, that "To assure batch uniformity and integrity of drug products, written procedures shall be established and followed that describe the in-process controls, and tests, or examinations to be conducted on appropriate samples of in-process materials of each batch. Such control procedures shall be established to monitor the output and to validate the performance of those manufacturing processes that may be responsible for causing variability in the characteristics of in-process material and the drug product * * *."

21 CFR 211.113(b) states that "Appropriate written procedures, designed to prevent microbiological contamination of drug products purporting to be sterile, shall be established and followed. Such procedures shall include validation of any sterilization process."

21 CFR 211.160(b) states that "Laboratory controls shall include the establishment of scientifically sound and appropriate specifications, standards, sampling plans, and test procedures designed to assure that components, drug product containers, closures, in-process materials, labeling, and drug products conform to appropriate standards of identity, strength, quality, and purity. Laboratory controls shall include: (1) Determination of conformance to appropriate written specifications for the acceptance of each lot within each shipment of components, drug product containers, closures, and labeling used in the manufacture, processing, packing, or holding of drug products. The specifications shall include a description of the sampling and testing procedures used. Samples shall be representative and adequately identified. Such procedures shall also require appropriate retesting of any component, drug product container, or closure that is subject to deterioration. (2) Determination of conformance to written specifications and a description of sampling and testing procedures for in-process materials. Such samples shall be representative and

properly identified. (3) Determination of conformance to written descriptions of sampling procedures and appropriate specifications for drug products. Such samples shall be representative and properly identified. (4) The calibration of instruments, apparatus, gauges, and recording devices at suitable intervals in accordance with an established written program containing specific directions, schedules, limits for accuracy and precision, and provisions for remedial action in the event accuracy and/or precision limits are not met. Instruments, apparatus, gauges, and recording devices not meeting established specifications shall not be used."

21 CFR 211.165(e) states that "The accuracy, sensitivity, specificity, and reproducibility of test methods employed by the firm shall be established and documented. Such validation and documentation may be accomplished in accordance with § 211.194(a)(2)."

21 CFR 211.192 states, in part, that "All drug product production and control records, including those for packaging and labeling, shall be reviewed and approved by the quality control unit to determine compliance with all established, approved written procedures before a batch is released or distributed * * *."

A.   Environmental Monitoring

1.   General Written Program

In aseptic processing, one of the most important laboratory controls is the environmental monitoring program. This program provides meaningful information on the quality of the aseptic processing environment (e.g., when a given batch is being manufactured) as well as environmental trends of ancillary clean areas. Environmental monitoring should promptly identify potential routes of contamination, allowing for implementation of corrections before product contamination occurs (211.42 and 211.113).

Evaluating the quality of air and surfaces in the cleanroom environment should start with a well-defined written program and scientifically sound methods. The monitoring program should cover all production shifts and include air, floors, walls, and equipment surfaces, including the critical surfaces that come in contact with the product, container, and closures. Written procedures should include a list of locations to be sampled. Sample timing, frequency, and location should be carefully selected based upon their relationship to the operation performed. Samples should be taken throughout the classified areas of the aseptic processing facility (e.g., aseptic corridors, gowning rooms) using scientifically sound sampling procedures. Sample sizes should be sufficient to optimize detection of environmental contaminants at levels that might be expected in a given clean area.

It is important that locations posing the most microbiological risk to the product be a key part of the program. It is especially important to monitor the microbiological quality of the critical area to determine whether or not aseptic conditions are maintained during filling and closing activities. Air and surface samples should be taken at the locations where significant activity or product exposure occurs during production. Critical surfaces that come in contact with the sterile product should remain sterile throughout an operation. When identifying critical sites to be sampled, consideration should be given to the points of contamination risk in a process, including factors such as difficulty of setup, length of processing time, and impact of interventions. Critical surface sampling should be performed at the conclusion of the aseptic processing operation to avoid direct contact with sterile surfaces during processing. Detection of microbial contamination on a critical site would not necessarily result in batch rejection. The

contaminated critical site sample should prompt an investigation of operational information and data that includes an awareness of the potential for a low incidence of false positives.

Environmental monitoring methods do not always recover microorganisms present in the sampled area. In particular, low-level contamination can be particularly difficult to detect. Because false negatives can occur, consecutive growth results are only one type of adverse trend. Increased incidence of contamination over a given period is an equal or more significant trend to be tracked. In the absence of any adverse trend, a single result above an action level should trigger an evaluation and a determination about whether remedial measures may be appropriate. In all room classes, remedial measures should be taken in response to unfavorable trends.

All environmental monitoring locations should be described in SOPs with sufficient detail to allow for reproducible sampling of a given location surveyed. Written SOPs should also address elements such as (1) frequency of sampling, (2) when the samples are taken (i.e., during or at the conclusion of operations), (3) duration of sampling, (4) sample size (e.g., surface area, air volume), (5) specific sampling equipment and techniques, (6) alert and action levels, and (7) appropriate response to deviations from alert or action levels.

2. *Establishing Levels and a Trending Program*

Microbiological monitoring levels should be established based on the relationship of the sampled location to the operation. The levels should be based on the need to maintain adequate microbiological control throughout the entire sterile manufacturing facility. One should also consider environmental monitoring data from historical databases, media fills, cleanroom qualification, and sanitization studies, in developing monitoring levels. Data from similar operations can also be helpful in setting action and alert levels, especially for a new operation.

Environmental monitoring data will provide information on the quality of the manufacturing environment. Each individual sample result should be evaluated for its significance by comparison to the alert or action levels. Averaging of results can mask unacceptable localized conditions. A result at the alert level urges attention to the approaching action conditions. A result at the action level should prompt a more thorough investigation. Written procedures should be established, detailing data review frequency and actions to be taken. The quality control unit should provide routine oversight of near-term (e.g., daily, weekly, monthly, quarterly) and long-term trends in environmental and personnel monitoring data.

Trend reports should include data generated by location, shift, room, operator, or other parameters. The quality control unit should be responsible for producing specialized data reports (e.g., a search on a particular isolate over a year period) with the goal of investigating results beyond established levels and identifying any appropriate follow-up actions. Significant changes in microbial flora should be considered in the review of the ongoing environmental monitoring data.

Written procedures should define the system whereby the most responsible managers are regularly informed and updated on trends and investigations.

3. *Disinfection Efficacy*

The suitability, efficacy, and limitations of disinfecting agents and procedures should be assessed. The effectiveness of these disinfectants and procedures should be measured by their ability to ensure that potential contaminants are adequately removed from surfaces.

To prevent introduction of contamination, disinfectants should be sterile, appropriately handled in suitable (e.g., sterile) containers and used for no longer than the predefined period specified by written procedures. Routinely used disinfectants should be effective against the normal microbial vegetative flora recovered from the facility. Many common disinfectants are ineffective against spores. For example, 70 percent isopropyl alcohol is ineffective against *Bacillus* spp. spores. Therefore, a sound disinfectant program also includes a sporicidal agent, used according to a written schedule and when environmental data suggest the presence of sporeforming organisms.

Disinfection procedures should be described in sufficient detail (e.g., preparation, work sequence, contact time) to enable reproducibility. Once the procedures are established, their adequacy should be evaluated using a routine environmental monitoring program. If indicated, microorganisms associated with adverse trends can be investigated as to their sensitivity to the disinfectants employed in the cleanroom in which the organisms were isolated.

4. *Monitoring Methods*

Acceptable methods for monitoring the microbiological quality of the environment include:

a. Surface Monitoring

Environmental monitoring involves sampling various surfaces for microbiological quality. For example, product contact surfaces, floors, walls, and equipment should be tested on a regular basis. Touch plates, swabs, and contact plates can be used for such tests.

b. Active Air Monitoring

Assessing microbial quality of air should involve the use of *active* devices including but not limited to impaction, centrifugal, and membrane (or gelatin) samplers. Each device has certain advantages and disadvantages, although all allow testing of the number of organisms per volume of air sampled. We recommend that such devices be used during each production shift to evaluate aseptic processing areas at carefully chosen locations. Manufacturers should be aware of a device's air monitoring capabilities, and the air sampler should be evaluated for its suitability for use in an aseptic environment based on collection efficiency, cleanability, ability to be sterilized, and disruption of unidirectional airflow.[20] Because devices vary, the user should assess the overall suitability of a monitoring device before it is placed into service. Manufacturers should ensure that such devices are calibrated and used according to appropriate procedures.

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[20] For example, the volume of air sampled should be sufficient to yield meaningful measurements of air quality in a given environment.

c.   Passive Air Monitoring (Settling Plates)

Another method is the use of passive air samplers, such as settling plates (petri dishes containing nutrient growth medium exposed to the environment). Because only microorganisms that settle onto the agar surface are detected, settling plates can be used as qualitative, or semi-quantitative, air monitors. Their value in critical areas will be enhanced by ensuring that plates are positioned in locations posing the greatest risk of product contamination. As part of methods validation, the quality control laboratory should evaluate what media exposure conditions optimize recovery of low levels of environmental isolates. Exposure conditions should preclude desiccation (e.g., caused by lengthy sampling periods and/or high airflows), which inhibits recovery of microorganisms. The data generated by passive air sampling can be useful when considered in combination with results from other types of air samples.

B.   Microbiological Media and Identification

Characterization of recovered microorganisms provides vital information for the environmental monitoring program. Environmental isolates often correlate with the contaminants found in a media fill or product sterility testing failure, and the overall environmental picture provides valuable information for an investigation. Monitoring critical and immediately surrounding clean areas as well as personnel should include routine identification of microorganisms to the species (or, where appropriate, genus) level. In some cases, environmental trending data have revealed migration of microorganisms into the aseptic processing room from either uncontrolled or lesser controlled areas. Establishing an adequate program for differentiating microorganisms in the lesser-controlled environments, such as Class 100,000 (ISO 8), can often be instrumental in detecting such trends. At minimum, the program should require species (or, where appropriate, genus) identification of microorganisms in these ancillary environments at frequent intervals to establish a valid, current database of contaminants present in the facility during processing (and to demonstrate that cleaning and sanitization procedures continue to be effective).

Genotypic methods have been shown to be more accurate and precise than traditional biochemical and phenotypic techniques. These methods are especially valuable for investigations into failures (e.g., sterility test; media fill contamination). However, appropriate biochemical and phenotypic methods can be used for the routine identification of isolates.

The goal of microbiological monitoring is to reproducibly detect microorganisms for purposes of monitoring the state of environmental control. Consistent methods will yield a database that allows for sound data comparisons and interpretations. The microbiological culture media used in environmental monitoring should be validated as capable of detecting fungi (i.e., yeasts and molds) as well as bacteria and incubated at appropriate conditions of time and temperature. Total aerobic bacterial count can be obtained by incubating at 30 to 35°C for 48 to 72 hours. Total combined yeast and mold count can generally be obtained by incubating at 20 to 25°C for 5 to 7 days.

Incoming lots of environmental monitoring media should be tested for their ability to reliably recover microorganisms. Growth promotion testing should be performed on all lots of prepared media. Where appropriate, inactivating agents should be used to prevent inhibition of growth by cleanroom disinfectants or product residuals (e.g., antibiotics).

C.   Prefiltration Bioburden

Manufacturing process controls should be designed to minimize the bioburden in the unfiltered product. In addition to increasing the challenge to the sterilizing filter, bioburden can contribute impurities (e.g., endotoxin) to, and lead to degradation of, the drug product. A prefiltration bioburden limit should be established.

D.   Alternate Microbiological Test Methods

Other suitable microbiological test methods (e.g., rapid test methods) can be considered for environmental monitoring, in-process control testing, and finished product release testing after it is demonstrated that the methods are equivalent or better than traditional methods (e.g.,USP).

E.   Particle Monitoring

Routine particle monitoring is useful in rapidly detecting significant deviations in air cleanliness from qualified processing norms (e.g., clean area classification). A result outside the established classification level at a given location should be investigated as to its cause. The extent of investigation should be consistent with the severity of the *excursion* and include an evaluation of trending data. Appropriate corrective action should be implemented, as necessary, to prevent future deviations.

See Section IV.A for additional guidance on particle monitoring.

XI. STERILITY TESTING

21 CFR 210.3(b)(21) states that *"Representative sample* means a sample that consists of a number of units that are drawn based on rational criteria such as random sampling and intended to assure that the sample accurately portrays the material being sampled."

21 CFR 211.110(a) states, in part, that "To assure batch uniformity and integrity of drug products, written procedures shall be established and followed that describe the in-process controls, and tests, or examinations to be conducted on appropriate samples of in-process materials of each batch. Such control procedures shall be established to monitor the output and to validate the performance of those manufacturing processes that may be responsible for causing variability in the characteristics of in-process material and the drug product."

21 CFR 211.160(b) states that "Laboratory controls shall include the establishment of scientifically sound and appropriate specifications, standards, sampling plans, and test procedures designed to assure that components, drug product containers, closures, in-process materials, labeling, and drug products conform to appropriate standards of identity, strength, quality, and purity. Laboratory controls shall include: (1) Determination of conformance to appropriate written specifications for the acceptance of each lot within each shipment of components, drug product containers, closures, and labeling used in the manufacture, processing, packing, or holding of drug products. The specifications shall include a description of the sampling and testing procedures used. Samples shall be representative and adequately identified. Such procedures shall also require appropriate retesting of any component, drug product container, or closure that is subject to deterioration. (2) Determination of conformance to written specifications and a description of sampling and testing procedures for in-process materials. Such samples shall be representative and properly identified. (3) Determination of conformance to written descriptions of sampling procedures and appropriate specifications for drug products. Such samples shall be representative and properly identified. (4) The calibration of instruments, apparatus, gauges, and recording devices at suitable intervals in accordance with an established written program containing specific directions, schedules, limits for accuracy and precision, and provisions for remedial action in the event accuracy and/or precision limits are not met. Instruments, apparatus, gauges, and recording devices not meeting established specifications shall not be used."

21 CFR 211.165(a) states, in part, that "For each batch of drug product, there shall be appropriate laboratory determination of satisfactory conformance to final specifications for the drug product, including the identity and strength of each active ingredient, prior to release * * *."

21 CFR 211.165(e) states that "The accuracy, sensitivity, specificity, and reproducibility of test methods employed by the firm shall be established and documented. Such validation and documentation may be accomplished in accordance with § 211.194(a)(2)."

21 CFR 211.167(a) states that "For each batch of drug product purporting to be sterile and/or pyrogen-free, there shall be appropriate laboratory testing to determine conformance to such requirements. The test procedures shall be in writing and shall be followed."

21 CFR 211.180(e) states, in part, that "Written records required by this part shall be maintained so that data therein can be used for evaluating, at least annually, the quality standards of each drug product to determine the need for changes in drug product specifications or manufacturing or control procedures * * *."

21 CFR 211.192 states that "All drug product production and control records, including those for packaging and labeling, shall be reviewed and approved by the quality control unit to determine compliance with all established, approved written procedures before a batch is released or distributed. Any unexplained discrepancy (including a percentage of theoretical yield exceeding the maximum or minimum percentages established in master production and control records) or the failure of a batch or any of its components to meet any of its specifications shall be thoroughly investigated, whether or not the batch has already been distributed. The investigation shall extend to other batches of the same drug product and other drug products that may have been associated with the specific failure or discrepancy. A written record of the investigation shall be made and shall include the conclusions and followup."

Certain aspects of sterility testing are of particular importance, including control of the testing environment, understanding the test limitations, and investigating manufacturing systems following a positive test.

The testing laboratory environment should employ facilities and controls comparable to those used for aseptic filling operations. Poor or deficient sterility test facilities or controls can result in test failure. If production facilities and controls are significantly better than those for sterility testing, the danger exists of mistakenly attributing a positive sterility test result to a faulty laboratory even when the product tested could have, in fact, been nonsterile. Therefore, a manufacturing deficiency may go undetected. The use of isolators for sterility testing minimizes the chance of a false positive test result.

A.   Microbiological Laboratory Controls

Sterility testing methods are required to be accurate and reproducible, in accordance with 211.194 and 211.165. USP <71> *"Sterility Tests"* is the principal source used for sterility testing methods, including information on test procedures and media.[21]

As a part of methods validation, appropriate microbiological challenge testing will demonstrate reproducibility of the method to reliably recover representative microorganisms. If growth is inhibited, modifications (e.g., increased dilution, additional membrane filter washes, addition of inactivating agents) to the test method should be implemented to optimize recovery. Ultimately, methods validation studies should demonstrate that the method does not provide an opportunity for false negatives.

It is essential that the media used to perform sterility testing be rendered sterile and demonstrated as growth promoting. Personnel performing sterility testing should be qualified and trained for the task. A written program should be in place to maintain updated training of personnel and confirm acceptable sterility testing practices.

B.   Sampling and Incubation

Sterility tests are limited in their ability to detect contamination because of the small sample size typically used. For example, as described by USP, statistical evaluations indicate that the sterility test sampling plan "only enables the detection of contamination in a lot in which 10% of the units are contaminated about nine times out of ten in making the test" (Ref. 13). To further illustrate, if a 10,000-unit lot with a 0.1 percent contamination level was sterility tested using 20 units, there is a 98 percent chance that the batch would pass the test.

It is important that the samples represent the entire batch and processing conditions. Samples should be taken:

- at the beginning, middle, and end of the aseptic processing operation
- in conjunction with processing interventions or excursions

Because of the limited sensitivity of the test, any positive result is considered a serious CGMP issue that should be thoroughly investigated.

C.   Investigation of Sterility Positives

Care should be taken in the performance of the sterility test to preclude any activity that allows for possible sample contamination. When microbial growth is observed, the lot should be considered nonsterile and an investigation conducted. An initial positive test would be invalid only in an instance in which microbial growth can be unequivocally ascribed to laboratory error.

Only if conclusive and documented evidence clearly shows that the contamination occurred as part of testing should a new test be performed. When available evidence is inconclusive, batches should be rejected as not conforming to sterility requirements.

After considering all relevant factors concerning the manufacture of the product and testing of the samples, the comprehensive written investigation should include specific conclusions and identify corrective actions. The investigation's persuasive evidence of the origin of the contamination should be based on at least the following:

1.   Identification (speciation) of the organism in the sterility test

Sterility test isolates should be identified to the species level. Microbiological monitoring data should be reviewed to determine if the organism is also found in laboratory and production environments, personnel, or product bioburden. Advanced identification methods (e.g., nucleic-acid based) are valuable for investigational purposes. When comparing results from environmental monitoring and sterility positives, both identifications should be performed using the same methodology.

2.   Record of laboratory tests and deviations

Review of laboratory deviation and investigation findings can help to eliminate or implicate the laboratory as the source of contamination. For example, if the organism is seldom found in the laboratory environment, product contamination is more likely than laboratory error. If the organism is found in laboratory and production environments, it can still indicate product contamination.

The proper handling of deviations is an essential aspect of laboratory control. When a deviation occurs during sterility testing, it should be documented, investigated, and remedied. If any deviation is considered to have compromised the integrity of the sterility test, the test should be invalidated immediately without incubation.

A sterility positive result can be viewed as indicative of production or laboratory problems, and the entire manufacturing process should be comprehensively investigated since such problems often can extend beyond a single batch. To more accurately monitor potential contamination sources, we recommend keeping separate trends by appropriate categories such as product, container type, filling line, sampling, and testing personnel. Where the degree of sterility test

sample manipulation is similar for a terminally sterilized product and an aseptically processed product, a higher rate of initial sterility failures for the latter should be taken as indicative of aseptic processing production problems.

Microbial monitoring of the aseptic area of the laboratory and personnel can also reveal trends that are informative. Upward trends in the microbial load in the aseptic area of the laboratory should be promptly investigated as to cause, and corrected. In some instances, such trends can appear to be more indicative of laboratory error as a possible source of a sterility test failure.

Where a laboratory has a good track record with respect to errors, this history can lower suspicion of the lab as a source of contamination since chances are higher that the contamination arose from production. However, the converse is not true. Specifically, where a laboratory has a poor track record, firms should not assume that the contamination is automatically more attributable to the laboratory and consequently overlook a genuine production problem. Accordingly, it is essential that all sterility positives be thoroughly investigated.

3. Monitoring of production area environment

Trend analysis of microorganisms in the critical and immediately adjacent areas is especially helpful in determining the source of contamination in a sterility failure investigation. Consideration of environmental microbial data should not be limited to results of monitoring the production environment for the lot, day, or shift associated with the suspect lot. For example, results showing little or no recovery of microorganisms can be misleading, especially when preceded or followed by a finding of an adverse trend or atypically high microbial counts. It is therefore important to look at both short- and long-term environmental trend analyses.

4. Monitoring Personnel

The review of data and associated trends from daily monitoring of personnel can provide important information indicating a route of contamination. The adequacy of personnel practices and training also merit significant review and consideration.

5. Product Presterilization Bioburden

We recommend review of trends in product bioburden and consideration of whether adverse bioburden trends have occurred.

6. Production record review

Complete batch and production control records should be reviewed to detect any signs of failures or anomalies that could have a bearing on product sterility. For example, the investigation should include elements such as:

- Events that could have impacted on the critical zone
- Batch and trending data that indicate whether utility and/or support systems are functioning properly. For instance, records of air quality monitoring for filling lines

could show a time at which there was improper air balance or an unusually high particle count.

- Whether construction or maintenance activities could have had an adverse impact

7. *Manufacturing history*

The manufacturing history of a product or similar products should be reviewed as part of the investigation. Past deviations, problems, or changes (e.g., process, components, equipment) are among the factors that can provide an indication of the origin of the problem.

XII.   BATCH RECORD REVIEW: PROCESS CONTROL DOCUMENTATION

21 CFR 211.100(a) states that "There shall be written procedures for production and process control designed to assure that the drug products have the identity, strength, quality, and purity they purport or are represented to possess. Such procedures shall include all requirements in this subpart. These written procedures, including any changes, shall be drafted, reviewed, and approved by the appropriate organizational units and reviewed and approved by the quality control unit."

21 CFR 211.100(b) states that "Written production and process control procedures shall be followed in the execution of the various production and process control functions and shall be documented at the time of performance. Any deviation from the written procedures shall be recorded and justified."

21 CFR 211.186 and 211.188 address, respectively, "Master production and control records" and "Batch production and control records."

21 CFR 211.192 states that "All drug product production and control records, including those for packaging and labeling, shall be reviewed and approved by the quality control unit to determine compliance with all established, approved written procedures before a batch is released or distributed. Any unexplained discrepancy (including a percentage of theoretical yield exceeding the maximum or minimum percentages established in master production and control records) or the failure of a batch or any of its components to meet any of its specifications shall be thoroughly investigated, whether or not the batch has already been distributed. The investigation shall extend to other batches of the same drug product and other drug products that may have been associated with the specific failure or discrepancy. A written record of the investigation shall be made and shall include the conclusions and followup."

Manufacturers should build process and environmental control activities into their aseptic processing operation. It is critical that these activities be maintained and strictly implemented on a daily basis. The requirement for review of all batch records and data for conformance with written procedures, operating parameters, and product specifications prior to arriving at the final release decision for an aseptically processed product calls for an overall review of process and system performance for that given cycle of manufacture. All in-process and laboratory control results must be included with the batch record documentation in accordance with section 211.188. Review of environmental and personnel monitoring data, as well as other data relating to acceptability of output from support systems (e.g., HEPA / HVAC, WFI, steam generator) and proper functioning of equipment (e.g., batch alarms report; integrity of various filters) are considered essential elements of the batch release decision.

While interventions and/or stoppages are normally recorded in the batch record, the manner of documenting these occurrences varies. In particular, line stoppages and any unplanned interventions should be sufficiently documented in batch records with the associated time and duration of the event. In addition to lengthened dwell time of sterile product elements in the critical area, an extensive intervention can increase contamination risk. Sterility failures have often been attributed to atypical or extensive interventions that have occurred as a response to an undesirable event during the aseptic process. Written procedures describing the need for line clearances in the event of certain interventions, such as machine adjustments and any repairs, should be established. Such interventions should be documented with more detail than minor events. Interventions that result in substantial activity near exposed product or container closures

or that last beyond a reasonable exposure time should, where appropriate, result in a local or full line clearance.

Any disruption in power supply, however momentary, that could affect product quality is a manufacturing deviation and must be included in batch records (211.100, 211.192).

APPENDIX 1: ASEPTIC PROCESSING ISOLATORS

Aseptic processing using isolation systems separates the external cleanroom environment from the aseptic processing line and minimizes its exposure to personnel. A well-designed positive pressure isolator, supported by adequate procedures for its maintenance, monitoring, and control, offers tangible advantages over traditional aseptic processing, including fewer opportunities for microbial contamination during processing. However, users should remain vigilant to potential sources of operational risk. Manufacturers should also be aware of the need to establish new procedures addressing issues unique to isolators.

A.   Maintenance

1. General

Maintenance of isolator systems differs in some significant respects from the traditional, non-isolated aseptic processing operations. Although no isolator forms an absolute seal, very high integrity can be achieved in a well-designed unit. However, a leak in certain components of the system can constitute a significant breach of integrity. The integrity of gloves, half-suits, and seams should receive daily attention and be addressed by a comprehensive preventative maintenance program. Replacement frequencies should be established in written procedures that ensure parts will be changed before they breakdown or degrade. Transfer systems, gaskets, and seals are among the other parts that should be covered by the maintenance program.

2. Glove Integrity

A faulty glove or sleeve (gauntlet) assembly represents a route of contamination and a critical breach of isolator integrity. A preventative maintenance program should be established. The choice of durable glove materials, coupled with a well-justified replacement frequency, are key aspects of good manufacturing practice to be addressed. With every use, gloves should be visually evaluated for any macroscopic physical defect. Physical integrity tests should also be performed routinely. A breach in glove integrity can be of serious consequence. The monitoring and maintenance program should identify and eliminate any glove lacking integrity and minimize the possibility of placing a sterile product at risk.

Due to the potential for microbial migration through microscopic holes in gloves and the lack of a highly sensitive glove integrity test, we recommend affording attention to the sanitary quality of the inner surface of the installed glove and to integrating the use of a second pair of thin gloves.

B.   Design

1. Airflow

There are two types of aseptic processing isolators: *open* and *closed*. Closed isolators employ connections with auxiliary equipment for material transfer. Open isolators have openings to the

surrounding environment that are carefully engineered to segregate the inner isolator environment from the surrounding room via overpressure.

Turbulent flow can be acceptable within closed isolators, which are normally compact in size and do not house processing lines. Other aseptic processing isolators employ unidirectional airflow that sweeps over and away from exposed sterile materials, avoiding any turbulence or stagnant airflow in the area of exposed sterilized materials, product, and container closures. In most sound designs, air showers over the critical area once and then is systematically exhausted from the enclosure. The air handling system should be capable of maintaining the requisite environmental conditions within the isolator.

2. Materials of Construction

As in any aseptic processing design, suitable materials should be chosen based on durability, as well as ease of cleaning and decontamination. For example, rigid wall construction incorporating stainless steel and glass materials is widely used.

3. Pressure Differential

Isolators that include an open portal should be designed to ensure complete physical separation from the external environment. A positive air pressure differential adequate to achieve this separation should be employed and supported by qualification studies. Positive air pressure differentials from the isolator to the surrounding environment have largely ranged from approximately 17.5 to 50 Pascals.[22] The appropriate minimum pressure differential established by a firm will depend on the system's design and, when applicable, its exit port. Air balance between the isolator and other direct interfaces (e.g., dry heat tunnel) should also be qualified.

The positive pressure differential should be coupled with an appropriately designed opening to the external environment to prevent potential ingress of surrounding room air by induction. Induction can result from local turbulent flow causing air swirls or pressure waves that might push extraneous particles into the isolator. Local Class 100 (ISO 5) protection at an opening is an example of a design provision that can provide a further barrier to the external environment.

4. Clean Area Classifications

The interior of the isolator should meet Class 100 (ISO 5) standards. The classification of the environment surrounding the isolator should be based on the design of its interfaces (e.g., transfer ports), as well as the number of transfers into and out of the isolator. A Class 100,000 (ISO 8) background is commonly used based on consideration of isolator design and manufacturing situations. An aseptic processing isolator should not be located in an unclassified room.

C. Transfer of Materials/Supplies

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[22] 0.07" to 0.20" water gauge

The ability to maintain integrity of a decontaminated isolator can be affected impacted by the design of transfer ports. Various adaptations, of differing capabilities, allow for the transfer of supplies into and out of the isolator.

Multiple material transfers are generally made during the processing of a batch. Frequently, transfers are performed via direct interface with manufacturing equipment. Properly maintained and operated rapid transfer ports (RTPs) are an effective transfer mechanism for aseptic transfer of materials into and out of isolators. Some transfer ports might have significant limitations, including marginal decontaminating capability (e.g., ultraviolet) or a design that has the potential to compromise isolation by allowing ingress of air from the surrounding room. In the latter case, localized HEPA-filtered unidirectional airflow cover in the area of such a port should be implemented. Isolators often include a *mousehole* or other exit port through which product is discharged, opening the isolator to the outside environment. Sufficient overpressure should be supplied and monitored on a continuous basis at this location to ensure that isolation is maintained.

D.   Decontamination

1. Surface Exposure

Decontamination procedures should ensure full exposure of all isolator surfaces to the chemical agent. The capability of a decontaminant to penetrate obstructed or covered surfaces is limited. For example, to facilitate contact with the decontaminant, the glove apparatus should be fully extended with glove fingers separated during the decontamination cycle. It is also important to clean the interior of the isolator per appropriate procedures to allow for a robust decontamination process.

2. Efficacy

The decontamination method should render the inner surfaces of the isolator free of viable microorganisms. Multiple available vaporized agents are suitable for achieving decontamination. Process development and validation studies should include a thorough determination of cycle capability. The characteristics of these agents generally preclude the reliable use of statistical methods (e.g., fraction negative) to determine process lethality (Ref. 13). An appropriate, quantified Biological Indicator (BI) challenge should be placed on various materials[23] and in many locations throughout the isolator, including difficult to reach areas. Cycles should be developed with an appropriate margin of extra kill to provide confidence in robustness of the decontamination processes. Normally, a four- to six-log reduction can be justified depending on the application. The specific BI spore titer used and the selection of BI placement sites should be justified. For example, demonstration of a four-log reduction should be sufficient for controlled, very low bioburden materials introduced into a transfer isolator, including wrapped sterile supplies that are briefly exposed to the surrounding cleanroom environment.

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[23] If the various isolator materials are thoroughly evaluated during cycle development, a firm might consider placing more focus on material texture and porosity during validation of the decontamination process.

The uniform distribution of a defined concentration of decontaminating agent should also be evaluated as part of these studies (Ref. 14). Chemical indicators may also be useful as a qualitative tool to show that the decontaminating agent reached a given location.

3. Frequency

The design of the interior and content of an isolator should provide for its frequent decontamination. When an isolator is used for multiple days between decontamination cycles, the frequency adopted should be justified. This frequency, established during validation studies, should be reevaluated and increased if production data indicate deterioration of the microbiological quality of the isolator environment.

A breach of isolator integrity should normally lead to a decontamination cycle. Integrity can be affected by power failures, valve failure, inadequate overpressure, holes in gloves and seams, or other leaks. Breaches of integrity should be investigated. If it is determined that the environment may have been compromised, any product potentially impacted by the breach should be rejected.

E.   Filling Line Sterilization

To ensure sterility of product contact surfaces from the start of each operation, the entire path of the sterile processing stream should be sterilized. In addition, aseptic processing equipment or ancillary supplies to be used within the isolator should be chosen based on their ability to withstand steam sterilization (or equivalent method). It is expected that materials that permit heat sterilization (e.g., SIP) will be rendered sterile by such methods. Where decontamination methods are used to render certain product contact surfaces free of viable organisms, a minimum of a six-log reduction should be demonstrated using a suitable biological indicator.

F.   Environmental Monitoring

An environmental monitoring program should be established that routinely ensures acceptable microbiological quality of air, surfaces, and gloves (or half-suits) as well as particle levels, within the isolator. Nutrient media should be cleaned off of surfaces following a contact plate sample. Air quality should be monitored periodically during each shift. For example, we recommend monitoring the exit port for particles to detect any unusual results. Media used for environmental monitoring should not be exposed to decontamination cycle residues, as recovery of microorganisms would be inhibited.

G.   Personnel

Although cleanroom apparel considerations are generally reduced in an isolator operation, the contamination risk contributed by manual factors can not be overlooked. Isolation processes generally include periodic or even frequent use of one or more gloves for aseptic manipulations and handling of material transfers into and out of the isolator. One should be aware that locations on gloves, sleeves, or half suits can be among the more difficult to reach places during decontamination, and glove integrity defects might not be promptly detected. Traditional aseptic processing vigilance remains critical, with an understanding that contaminated isolator gloves

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can lead to product nonsterility. Accordingly, meticulous aseptic technique standards must be observed (211.113), including appropriate use of sterile tools for manipulations.

APPENDIX 2: BLOW-FILL- SEAL TECHNOLOGY

Blow-fill-seal (BFS) technology is an automated process by which containers are formed, filled, and sealed in a continuous operation. This manufacturing technology includes economies in container closure processing and reduced human intervention and is often used for filling and packaging ophthalmics, respiratory care products, and, less frequently, injectables. This appendix discusses some of the critical control points of this technology. Except where otherwise noted below, the aseptic processing standards discussed elsewhere in this document should apply to blow-fill-seal technology.

A.   Equipment Design and Air Quality

Most BFS machines operate using the following steps.

- Heat a plastic polymer resin
- Extrude it to form a parison (a tubular form of the hot resin)
- Cut the parison with a high-temperature knife
- Move the parison under the blow-fill needle (mandrel)
- Inflate it to the shape of the mold walls
- Fill the formed container with the liquid product
- Remove the mandrel
- Seal

Throughout this operation, sterile-air is used, for example, to form the parison and inflate it prior to filling. In most operations, the three steps with the greatest potential for exposure to particle contamination and/or surrounding air are those in which (1) the parison is cut, (2) the parison is moved under the blow-fill mandrel, and (3) the mandrel is removed (just prior to sealing).

BFS machinery and its surrounding barriers should be designed to prevent the potential for extraneous contamination. As with any aseptic processing operation, it is critical that product contact surfaces be sterile. A validated steam-in-place cycle, or equivalent process, should be used to sterilize the equipment path through which the product is conveyed. In addition, any other surface that represents a potential contamination risk to the sterile product should be sterile.

The classified environment surrounding BFS machinery should generally meet Class 100,000 (ISO 8), or better, standards, depending on the design of the BFS machinery and the surrounding room. HEPA-filtered or sterile air provided by membrane filters should be used during the steps when sterile products or materials are exposed (e.g., parison formation, container molding or filling steps). Air in the critical area should meet Class 100 (ISO 5) microbiological standards during operations. A well-designed BFS system should also normally achieve Class 100 (ISO 5) airborne particle levels. Only personnel who have been qualified and appropriately gowned should enter the classified environment surrounding the BFS machinery. Refer to Section V of this document for guidance on personnel training, qualification, and monitoring.

BFS equipment design typically calls for use of specialized measures to reduce particle levels that can contaminate the exposed product. In contrast to nonpharmaceutical applications using

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BFS machinery, control of air quality (i.e., particles) is critical for sterile drug product manufacture. Particles generated during the plastic extrusion, cutting, and sealing processes should be controlled. Provisions for carefully controlled airflow can protect the product by forcing generated particles outward while preventing any ingress from the adjacent environment. Furthermore, equipment designs that separate the filling zone from the surrounding environment provide additional product protection. Barriers, pressure vacuums, microenvironments, and appropriately directed high velocities of sterile air have been found useful in preventing contamination (Ref. 15). Smoke studies and multi-location particle data can provide valuable information when performing qualification studies to assess whether proper particle control dynamics have been achieved throughout the critical area.

In addition to suitable design, it is important to establish an adequate preventative maintenance program. For example, because of its potential to contaminate the sterile drug product, the integrity of the cooling, heating and other utility systems associated with the BFS machine should be maintained and routinely monitored.

B.   Validation/Qualification

Advantages of BFS processing are known to include rapid container closure processing and minimized aseptic interventions. However, only a properly functioning process can realize these advantages. We recommend affording special attention to setup, troubleshooting of equipment, and related aseptic personnel procedures. Equipment sterilization, media fills, polymer extrusion/sterilization, product-plastic compatibility, forming and sealing integrity, and unit weight variation are among the key issues to address in validation and qualification studies.

Data gathered during such studies should ensure that BFS containers are sterile and, if used for parenteral drugs, nonpyrogenic. This can generally be achieved by validating that time temperature conditions of the extrusion process are effective against endotoxin or spore challenges in the polymeric material.

The choice of appropriate polymer material for a BFS operation includes assessing if a material is pharmaceutical grade, safe, pure, and passes appropriate criteria (Ref. 17) for plastics. Polymer suppliers should be qualified and monitored for raw material quality.

C.   Batch Monitoring and Control

Various in-process control parameters (e.g., container weight variation, fill weight, leakers, air pressure) provide information to monitor and facilitate ongoing process control. It is essential to monitor the microbial air quality. Samples should be taken according to a comprehensive sampling plan that provides data representative of the entire filling operation. Continuous monitoring of particles can provide valuable data relative to the control of a blow-fill-seal operation.

Container closure defects can be a major problem in control of a BFS operation. It is critical that the operation be designed and set-up to uniformly manufacture integral units. As a final measure, the inspection of each unit of a batch should include a reliable, sensitive, final product examination that is capable of identifying defective units (e.g., *leakers*). Significant defects due

to heat or mechanical problems, such as wall thickness, container or closure interface deficiencies, poorly formed closures, or other deviations should be investigated in accordance with §§ 211.100 and 211.192.

APPENDIX 3: PROCESSING PRIOR TO FILLING AND SEALING OPERATIONS

The purpose of this appendix is to supplement the guidance provided in this document with information on products regulated by CBER or CDER that are subject to aseptic processing at points early in the manufacturing process, or that require aseptic processing through the entire manufacturing process because it is impossible to sterile filter the final drug product. The scope of this appendix includes aseptic processing activities that take place prior to the filling and sealing of the finished drug product. Special considerations include those for:

A.   Aseptic processing from early manufacturing steps

Some products undergo aseptic processing at some or all manufacturing steps preceding the final product closing step. With other products, there is a point in the process after which they can no longer be rendered sterile by filtration. In such cases, the product would be handled aseptically at all steps subsequent to sterile filtration. In other instances, the final drug product cannot be sterile-filtered and, therefore, each component in the formulation would be rendered sterile and mixed aseptically. For example, products containing aluminum adjuvant are formulated aseptically because once they are alum adsorbed, they cannot be sterile-filtered.

When a product is processed aseptically from the early stages, the product and all components or other additions are rendered sterile prior to entering the manufacturing process. It is critical that all transfers, transports, and storage stages be carefully controlled at each step of the process to maintain sterility of the product. In some cases, bulk drug substances or products should be tested for sterility.[24]

Procedures (e.g., aseptic connection) that expose a product or product contact surfaces should be performed under unidirectional airflow in a Class 100 (ISO 5) environment. The environment of the room surrounding the Class 100 (ISO 5) environment should be Class 10,000 (ISO 7) or better. Microbiological and airborne particle monitoring should be performed during operations. Microbial surface monitoring should be performed at the end of operations, but prior to cleaning. Personnel monitoring should be performed in association with operations.

Process simulation studies covering the steps preceding filling and sealing should be designed to incorporate all conditions, product manipulations, and interventions that could impact on the sterility of the product. The process simulation, from the early process steps, should demonstrate that process controls are adequate to protect the product during manufacturing. These studies should incorporate all product manipulations, additions, and procedures involving exposure of product contact surfaces to the environment. The studies should include worst-case conditions such as maximum duration of open operations and maximum number of participating operators. However, the process simulations do not need to mimic total manufacturing time if the manipulations that occur during manufacturing are adequately represented.

It is also important that process simulations incorporate storage of sterile bulk drug substances or product and transport to other manufacturing areas. For instance, there should be assurance of bulk vessel integrity for specified holding times. The transport of sterile bulk tanks or other containers should be simulated as part of the media fill. Please refer to Section IX.A for more

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[24] See 21 CFR 610.12 for general biological product standards for sterility.

guidance on media simulation studies. Process simulation studies for the formulation stage should be performed at least twice per year.

B.   Aseptic processing of cellular therapy products and cell-derived products

Cellular therapy and some cell-derived products (e.g., lysates, semi-purified extracts) represent a subset of the products that cannot be filter-sterilized and therefore undergo aseptic manipulations throughout the manufacturing process. Where possible, closed systems should be used during manufacturing. Cellular therapy products often have short processing times at each manufacturing stage, particularly between the harvest, formulation of the final product, and product release. These products are frequently released from the manufacturing facility and administered to patients before final product sterility testing results are available. In situations where results of final sterility testing are not available before the product is administered, additional controls and testing should be considered. For example, additional sterility tests can be performed at intermediate stages of manufacture, such as after the last manipulation of the product prior to harvest. Other tests that may indicate microbial contamination, such as microscopic examination, Gram stain (or other bacterial and fungal stain), and endotoxin testing should be performed and meet acceptance criteria prior to product release.

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RELEVANT GUIDANCE DOCUMENTS

Some relevant FDA guidance documents include:

- Guidance for the Submission of Documentation for Sterilization Process Validation in Applications for Human and Veterinary Drug Products

- Guideline for Validation of Limulus Amebocyte Lysate Test as an End Product Endotoxin Test for Human and Animal Parenteral Drugs, Biological Products, and Medical Devices

- Guide to Inspections of Lyophilization of Parenterals

- Guide to Inspections of High Purity Water Systems

- Guide To Inspections of Microbiological Pharmaceutical Quality Control Laboratories

- Guide To Inspections of Sterile Drug Substance Manufacturers

- Pyrogens: Still a Danger; (Inspection Technical Guide)

- Bacterial Endotoxins/Pyrogens; (Inspection Technical Guide)

- Heat Exchangers to Avoid Contamination; (Inspection Technical Guide)

- Compliance Program Guidance Manual 7356.002 A, Sterile Drug Process Inspections

- ICH Q5A, Guidance on Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin

- See also the draft guidance Container and Closure Integrity Testing in Lieu of Sterility Testing as a Component of the Stability Protocol for Sterile Products, which was issued in 1998. Once final, it will represent the Agency's thinking on this topic.

GLOSSARY

Air lock- A small room with interlocked doors, constructed to maintain air pressure control between adjoining rooms (generally with different air cleanliness standards). The intent of an aseptic processing airlock is to preclude ingress of particulate matter and microorganism contamination from a lesser controlled area.

Alert Level- An established microbial or airborne particle level giving early warning of potential drift from normal operating conditions and triggers appropriate scrutiny and follow-up to address the potential problem. Alert levels are always lower than action levels.

Action Level- An established microbial or airborne particle level that, when exceeded, should trigger appropriate investigation and corrective action based on the investigation.

Aseptic Manufacturing Area- The classified part of a facility that includes the aseptic processing room and ancillary cleanrooms. For purposes of this document, this term is synonymous with "aseptic processing facility" as used in the segregated segment context.

Aseptic Processing Facility- A building, or segregated segment of it, containing cleanrooms in which air supply, materials, and equipment are regulated to control microbial and particle contamination.

Aseptic Processing Room- A room in which one or more aseptic activities or processes is performed.

Asepsis- A state of control attained by using an aseptic work area and performing activities in a manner that precludes microbiological contamination of the exposed sterile product.

Bioburden- The total number of microorganisms associated with a specific item prior to sterilization.

Barrier- A physical partition that affords aseptic processing area (ISO 5) protection by partially separating it from the surrounding area.

Biological Indicator (BI)- A population of microorganisms inoculated onto a suitable medium (e.g., solution, container or closure) and placed within appropriate sterilizer load locations to determine the sterilization cycle efficacy of a physical or chemical process. The *challenge microorganism* is selected based upon its resistance to the given process. Incoming lot D-value and microbiological count define the quality of the BI.

Clean Area- An area with defined particle and microbiological cleanliness standards.

Cleanroom- A room designed, maintained, and controlled to prevent particle and microbiological contamination of drug products. Such a room is assigned and reproducibly meets an appropriate air cleanliness classification.

Component- Any ingredient intended for use in the manufacture of a drug product, including those that may not appear in the final drug product.

Colony Forming Unit (CFU)- A microbiological term that describes the formation of a single macroscopic colony after the introduction of one or more microorganisms to microbiological growth media. One colony forming unit is expressed as 1 CFU.

Critical Area - An area designed to maintain sterility of sterile materials. Sterilized product, containers, closures, and equipment may be exposed in critical areas.

Clean Zone- See Clean Area.

Critical surfaces- Surfaces that may come into contact with or directly affect a sterilized product or its containers or closures. Critical surfaces are rendered sterile prior to the start of the manufacturing operation, and sterility is maintained throughout processing.

Decontamination- A process that eliminates viable bioburden via use of sporicidal chemical agents.

Disinfection- Process by which surface bioburden is reduced to a safe level or eliminated. Some disinfection agents are effective only against vegetative microbes, while others possess additional capability to effectively kill bacterial and fungal spores.

Depyrogenation- A process used to destroy or remove pyrogens (e.g., endotoxin).

D value- The time (in minutes) of exposure at a given temperature that causes a one-log or 90 percent reduction in the population of a specific microorganism.

Dynamic- Conditions relating to clean area classification under conditions of normal production.

Endotoxin- A pyrogenic product (e.g., lipopolysaccharide) present in the bacterial cell wall. Endotoxin can lead to reactions in patients receiving injections ranging from fever to death.

Gowning Qualification- A program that establishes, both initially and on a periodic basis, the capability of an individual to don the complete sterile gown in an aseptic manner.

HEPA filter- High efficiency particulate air filter with minimum 0.3 μm particle retaining efficiency of 99.97 percent.

HVAC- Heating, ventilation, and air conditioning.

Intervention- An aseptic manipulation or activity that occurs at the critical area.

Isolator- A decontaminated unit, supplied with Class 100 (ISO 5) or higher air quality, that provides uncompromised, continuous isolation of its interior from the external environment (e.g., surrounding cleanroom air and personnel). There are two major types of isolators:

Closed isolator systems exclude external contamination from the isolator's interior by accomplishing material transfer via aseptic connection to auxiliary equipment, rather than use of openings to the surrounding environment. Closed systems remain sealed throughout operations.

Open isolator systems are designed to allow for the continuous or semi-continuous ingress and/or egress of materials during operations through one or more openings. Openings are engineered (e.g., using continuous overpressure) to exclude the entry of external contamination into the isolator.

Laminar flow- An airflow moving in a single direction and in parallel layers at constant velocity from the beginning to the end of a straight line vector.

Operator- Any individual participating in the aseptic processing operation, including line set-up, filler, maintenance, or other personnel associated with aseptic line activities.

Overkill sterilization process- A process that is sufficient to provide at least a 12 log reduction of microorganisms having a minimum D value of 1 minute.

Pyrogen- A substance that induces a febrile reaction in a patient.

Sterile Product- For purposes of this guidance, *sterile product* refers to one or more of the elements exposed to aseptic conditions and ultimately making up the sterile finished drug product. These elements include the containers, closures, and components of the finished drug product.

Sterilizing grade filter- A filter that, when appropriately validated, will remove all microorganisms from a fluid stream, producing a sterile effluent.

Quality Control Unit- An organizational element with authority and responsibility as defined by 211.22.

Unidirectional flow- An airflow moving in a single direction, in a robust and uniform manner, and at sufficient speed to reproducibly sweep particles away from the critical processing or testing area.

Terminal sterilization- The application of a lethal agent to sealed, finished drug products for the purpose of achieving a predetermined sterility assurance level (SAL) of usually less than 10^{-6} (i.e., a probability of a nonsterile unit of greater than one in a million).

ULPA filter- Ultra-low penetration air filter with minimum 0.3 μm particle retaining efficiency of 99.999 percent.

Validation- Establishing documented evidence that provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes.

<u>Worst case</u>- A set of conditions encompassing upper and lower processing limits and circumstances, including those within standard operating procedures, that pose the greatest chance of process or product failure (when compared to ideal conditions). Such conditions do not necessarily induce product or process failure.